Journal of Physiology (1992), **447**, *pp.* 49–66
With 7 figures
Printed in Great Britain

ELECTRICAL BEHAVIOUR OF MYENTERIC NEURONES IN THE GASTRIC ANTRUM OF THE GUINEA-PIG

BY J. F. TACK* AND J. D. WOOD

From the Department of Physiology, College of Medicine, The Ohio State University, 4196 Graves Hall, 333 West 10th Avenue, Columbus, OH 43210-1239, USA

(*Received 16 April 1991*)

SUMMARY

1. Intracellular microelectrodes were used to study the electrical behaviour of ganglion cells in the myenteric plexus of the antrum of the guinea-pig stomach. In the absence of any information on antral myenteric neurones, the aim was to characterize the electrical behaviour and identify biophysical properties of the neurones that could be related to specialized organization of the neural microcircuits in this physiologically important division of the stomach.

2. Myenteric neurones in the gastric antrum were classified into four subtypes based on electrophysiological properties. These were gastric I, II, III and AH/type 2 neurones. Gastric I neurones were characterized by repetitive spike discharge during intraneuronal injection of depolarizing current, by higher input resistances and by lower resting membrane potentials than the other cell types. Gastric II neurones did not discharge repetitively. They discharged one or two spikes only at the beginning of depolarizing current pulses. Gastric III neurones did not discharge action potentials in response to depolarizing pulses. These neurones had higher membrane potentials and lower input resistances than the other types. A fourth type of neurone discharged one or more spikes during depolarizing current pulses and had long-lasting hyperpolarizing after-potentials associated with the spikes. The behaviour of these neurones was like AH/type 2 neurones found elsewhere in the enteric nervous system.

3. Action potentials in gastric I and II neurones were abolished by tetrodotoxin. Spikes of the AH/type 2 cells were not abolished by tetrodotoxin due to a calcium component of the inward current. Application of tetraethylammonium broadened the spikes. This was reversed by removal of Ca^{2+} from the bathing medium.

4. The hyperpolarizing after-potentials of AH/type 2 neurones were suppressed by removal of Ca^{2+} from the bathing medium. Treatment with 4-aminopyridine decreased the amplitude and duration of the after-hyperpolarization, whereas tetraethylammonium increased the duration and amplitude of the after-potentials. The hyperpolarizing after-potentials were unaffected by apamin.

5. Elevation of cyclic 3′,5′-adenosine monophosphate by forskolin resulted in

* Present address: Laboratorium Gastrointestinale, Fysiopathologie, Katholieke Universiteit, Leuven, Herestraat 49, B-3000 Leuven, Belgium.

MS 9306

excitation of all AH/type 2 neurones and some of the gastric III cells. Gastric I and II neurones were unaffected.

6. The electrophysiological behaviour of myenteric neurones in the antrum was similar in some respects and different in others from neurones in the gastric corpus and the small and large intestine of the same animal. The differences may reflect distinct organization of the microcircuits for the specialized neural control of the effector functions which characterize the gastric antrum.

INTRODUCTION

The myenteric plexus is a significant part of the neural integrative system that programs and co-ordinates a variety of muscle motility and mucosal secretory patterns in different regions of the gastrointestinal tract (Wood, 1987*a*, *b*). Initially, the small intestine was the only part of the gut where detailed information on the cellular neurophysiology of myenteric ganglion cells was available. The electrical and synaptic properties of myenteric neurones in the small intestine have been reviewed by several authors (Gershon & Erde, 1981; North, 1986; Wood, 1987*a*, *b*, 1989). Recent reports have described the electrical and synaptic behaviour in the myenteric plexuses of the colon, the terminal large intestine and the gastric corpus (Furukawa, Taylor & Bywater, 1986; Brookes, Ewart & Wingate, 1987; Wade & Wood, 1988*a*, *b*; Schemann & Wood, 1989*a*, *b*; Tamura & Wood, 1989). In each of these regions, distinctive electrical and synaptic properties of the myenteric neurones were found.

The myenteric plexus of the gastric antrum is involved in functions associated with interdigestive gastroduodenal motility, gastric emptying and gastroduodenal co-ordination (Deloof & Rousseau, 1985; Allescher, Daniel, Dent, Fox & Kostolanska, 1988). As in other specialized divisions of the digestive tract, the electrical and synaptic properties of the myenteric neurones in the antrum are likely to reflect the specific functions of this highly specialized region. The present study was undertaken to characterize the electrophysiological behaviour of myenteric neurones in the gastric antrum and compare the results with the properties of myenteric neurones in other regions of the gut. An abstract of this work has been published (Tack & Wood, 1990).

METHODS

Male guinea-pigs (400–600 g) were stunned by a blow to the head and exsanguinated from the cervical vessels. After removing the stomach, the segment distal to the incisura angularis was placed in ice-cold Krebs solution in a dissection dish. It was pinned flat mucosal side up to Sylgard 184 encapsulating resin (Dow Corning, Midland, MI, USA). Fine forceps were used to remove the mucosa and inner muscle layers and expose the myenteric plexus on the longitudinal muscle layer.

A $1{\cdot}5 \times 1$ cm segment of the preparation was then transferred and pinned to Sylgard resin at the bottom of a 1·5 ml recording chamber. The chamber was continuously perfused at a rate of 10–15 ml/min with Krebs solution at 37 °C and gassed with 95% O_2–5% CO_2 to buffer at a pH of 7·38. The composition of the Krebs solution was (mM): NaCl, 120·9; KCl, 5·9; $MgCl_2$, 1·2; NaH_2PO_4, 1·2; $NaHCO_3$, 14·4; $CaCl_2$, 2·5; glucose, 11·5. Modified high Mg^{2+}–low Ca^{2+} Krebs solution contained 1·25 mM-Ca^{2+} and 16 mM-Mg^{2+}.

Myenteric ganglia were visualized with a compound microscope with differential interference contrast optics and epilumination. Individual ganglia were immobilized with L-shaped stainless-steel wires (Wood & Mayer, 1978). In addition, in about 60% of the experiments, smooth muscle contraction was prevented by application of nicardipine (0·1–0·2 M) in the superfusion solution.

Conventional intracellular recording methods with glass microelectrodes filled with 3 M-KCl and having resistances of 80–140 MΩ were used. The amplifier (Dagan 8100, Dagan Instruments, Minneapolis, MN, USA) was equipped with negative capacity compensation and bridge circuitry for injecting electrical current through the microelectrode. Rectangular electrical current pulses with durations of 1–200 ms were injected through the microelectrode and were driven by Grass SD9 stimulators (Grass Instruments, Quincy, MA, USA).

Electrophysiological data were recorded on videotape (A. R. Vetter Co., Rebersburg, PA, USA) and events with slow time courses were replayed on a strip chart recorder (Gould 2400, Gould Inst., Cleveland, OH, USA). Fast events were analysed with digital and analogue oscilloscopes (Tektronics 5113, Tektronics Inc., Beaverton, OR, USA).

Actions of pharmacologically active agents were studied by micropressure ejection from fine-tipped pipettes (< 10 μm) manipulated in close proximity to the impaled neurone. Pressure pulses of nitrogen with predetermined force and duration were applied to the micropipettes through electronically controlled solenoid valves (Picospritzer II, General Valve Co, Fairfield, NJ, USA).

Tetrodotoxin with citrate buffer was dissolved in distilled water. Tetraethylammonium and apamin were prepared fresh daily in Krebs solution. 4-Aminopyridine was dissolved in 0·1 M-HCl and the pH adjusted to 7·38 with NaOH. These agents were obtained from Sigma Biochemicals, St Louis, MO, USA. The water-soluble form of forskolin was obtained from Calbiochem–Behring, San Diego, CA, USA and dissolved in Krebs solution.

Resting membrane potentials were determined after 5 min of stable impalement. Input resistances were estimated according to the method of Grafe, Mayer & Wood (1980). Time constants were estimated as the time required for electronic potentials to reach 63 % of recovery towards the resting potential. All values are given as the mean ± standard error of the mean. Statistical differences were determined by one-way analysis of variance. Probability < 0·05 was accepted as significant.

RESULTS

Studies were done in 370 neurones from 146 guinea-pigs. Impalements lasted from 30 min to 9 h. Four types of neurones were distinguished by electrophysiological behaviour. Of 370 neurones, 283 had behaviour comparable to neurones in the gastric corpus (Schemann & Wood, 1989*a*). Consequently, we adopted the same classification scheme and referred to the three types of neurones as gastric I, II or III. Eighty-seven neurones did not fit in this classification. These neurones were similar to the AH/type 2 neurones described elsewhere in the enteric nervous system (Wood, 1987*b*, 1989). Therefore, this terminology is used for this kind of neurone in the antrum. Table 1 gives proportions for each type of neurone.

Gastric I neurones

Gastric I neurones were the most excitable of the four types. During intrasomatic injection of suprathreshold depolarizing current pulses of 100–200 ms duration, neurones in this subpopulation responded by repetitive discharge of action potentials (Fig. 1). The frequency of spike discharge increased in direct relation to the magnitude of the injected current pulse. The maximum rate of discharge during the strongest current pulses was 100 Hz. During depolarizations lasting up to 30 s, discharge was not sustained beyond the first 200–300 ms. During the repetitive discharge, the frequency of spike discharge and the rates of rise and fall of the action potential progressively decreased. For eight neurones at the maximal rate of discharge during a 200 ms pulse, the mean dV/dt for the rising phase of the last spike was 52 ± 13 % of the first spike and the mean dV/dt for the falling phase of the last spike was 79 ± 14 % of the first spike. Table 1 lists specific data on electrical behaviour of gastric I neurones.

Gastric II neurones

Gastric II neurones were less excitable than the gastric I type. This was evident as discharge of only one or two action potentials at the onset of depolarizing current pulses irrespective of the current strength (Fig. 1). Mean resting potential for gastric II neurones was significantly higher than gastric I neurones. The input resistance, membrane time constant and the threshold for spike discharge were significantly lower than for gastric I neurones (Tables 1 and 2).

TABLE 1. Electrophysiological behaviour of myenteric neurones in the guinea-pig gastric antrum

	Gastric I	Gastric II	Gastric III	Gastric AH/type 2
Percentage of 370 neurones	19·2	47·8	9·5	23·5
Repetitive discharge	(+)	(−)	(−)	(+)*
Anodal break	(+) 18·3%	(+) 3·2%	(−)	(−)
Resting potential (mV)	$-58·1 \pm 1·2$ $n = 67$	$-63·7 \pm 1·0$ $n = 129$	$-70·8 \pm 1·8$ $n = 21$	$-62·6 \pm 1·5$ $n = 80$
Input resistance (mΩ)	$169·4 \pm 15·8$ $n = 23$	$101·9 \pm 5·4$ $n = 66$	$50·3 \pm 6·3$ $n = 17$	$109·6 \pm 9·6$ $n = 46$
Time constant (ms)	$5·6 \pm 0·3$ $n = 33$	$2·6 \pm 0·2$ $n = 43$	$1·6 \pm 0·3$ $n = 19$	$3·7 \pm 0·2$ $n = 28$

* Repetitive discharge occurred only during a special case of synaptic activation.

Gastric III cells

Gastric III cells, unlike gastric I and II neurones, were never induced to discharge action potentials in response to depolarizing current pulses. They could however be identified as neurones on the basis of stimulus-evoked synaptic potentials or electronic invasion of the somal membrane by spikes evoked in the neurites (Tack & Wood, 1991).

The mean resting potential of gastric III neurones was significantly higher than gastric I and II neurones. The input resistance and time constant were significantly lower than in gastric I and II neurones (Tables 1 and 2).

AH/type 2 neurones

AH/type 2 neurones had low excitability, as evidenced by discharge of one or a few action potentials at the beginning of depolarizing current pulses (Fig. 1). Some AH/type 2 neurones with enhanced excitability were encountered and these are described below. The state of enhanced excitability did not reflect the resting condition of the neurone. The enhanced excitability and repetitive spike discharge in these cases were attributed to activation of the neurone by on-going release of excitatory neurotransmitters.

AH/type 2 neurones had resting potentials that were significantly higher than in gastric I cells and significantly lower than gastric III cells. The threshold for spike discharge was significantly lower than in gastric I cells, whereas both the input resistance and the membrane time constant were significantly lower than in gastric

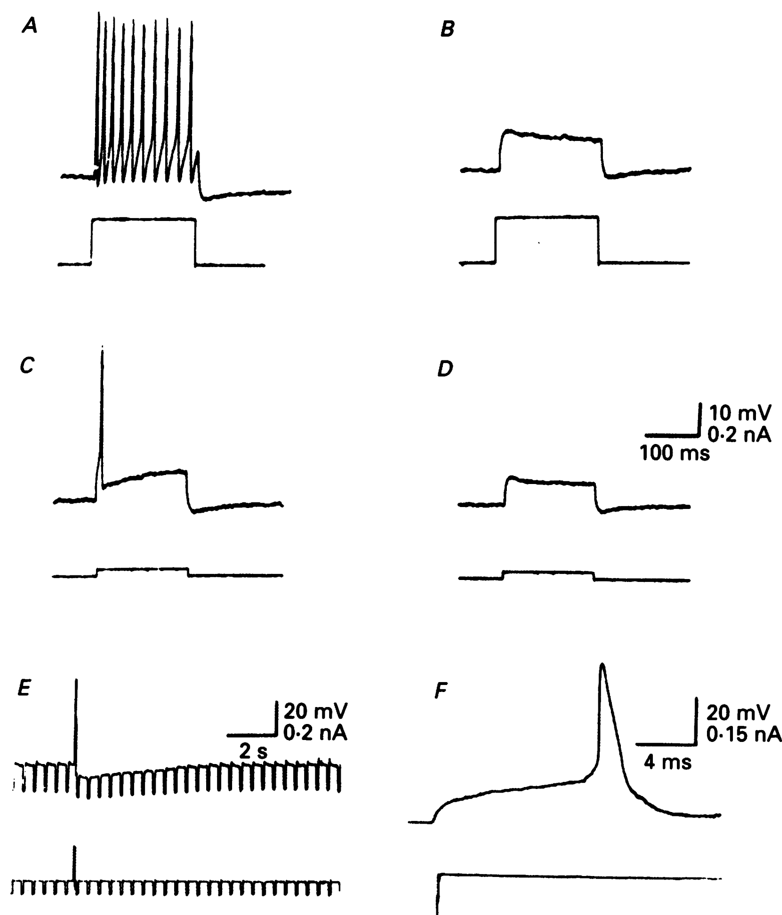

Fig. 1. Electrophysiological behaviour of neurones in the myenteric plexus of the guinea-pig gastric antrum. *A*, gastric I neurone discharged repetitively during a depolarizing current pulse. *B*, spike discharge was blocked by 0·1 μM-tetrodotoxin. *C*, gastric II neurone discharged only one action potential at the onset of depolarizing current pulses. *D*, spike discharge was blocked by 0·1 μM-tetrodotoxin. *E*, in an AH/type 2 neurone, the action potentials were associated with a long-lasting hyperpolarizing after-potential. Decreased amplitude of electrotonic potentials produced by repetitive injection of hyperpolarizing current pulses reflected decreased input resistance during the after-potential. *F*, action potential of the AH/type 2 neurone in *E* recorded with an expanded time base. AH/type 2 neurones usually discharged one action potential at the onset of depolarizing current pulses. A 'shoulder' on the falling phase of the action potential was characteristic for AH/type 2 neurones. Upper trace is transmembrane voltage; lower trace is injected current.

I cells ($P < 0{\cdot}001$) and significantly higher than in gastric III neurones (Tables 1 and 2).

Most of the AH/type 2 neurones discharged only one or two action potentials during depolarizing current pulses. However, during longer impalements, ten AH/type 2 neurones became 'activated' and displayed enhanced excitability. These

TABLE 2. Characteristics of action potentials in myenteric neurones of the guinea-pig gastric antrum

	Gastric I	Gastric II	Gastric AH/type 2
Spike threshold (mv)	$-38{\cdot}8 \pm 0{\cdot}8$ ($n = 23$)	$-30{\cdot}4 \pm 0{\cdot}7$ ($n = 23$)	$-31{\cdot}4 \pm 1{\cdot}0$ ($n = 16$)
dV/dt (V/s)	$90{\cdot}8 \pm 22{\cdot}9$* $67{\cdot}4 \pm 12{\cdot}6$† ($n = 23$)	$80{\cdot}4 \pm 21{\cdot}3$* $59{\cdot}7 \pm 21{\cdot}5$† ($n = 54$)	$92{\cdot}4 \pm 26{\cdot}2$* $53{\cdot}6 \pm 13{\cdot}5$† ($n = 16$)
	Gastric I & II		Gastric AH/type 2
Spike duration (ms)	$1{\cdot}2 \pm 0{\cdot}1$ ($n = 23$)		$2{\cdot}5 \pm 0{\cdot}1$ ($n = 16$)
AH‡ amplitude (mV)	$0{\cdot}186 \pm 0{\cdot}02$ ($n = 28$)		$11{\cdot}7 \pm 1{\cdot}0$ ($n = 27$)
AH‡ duration (s)	$0{\cdot}186 \pm 0{\cdot}02$ ($n = 228$)		$7{\cdot}2 \pm 1{\cdot}1$ ($n = 27$)
AH‡ decay constant (s)	$0{\cdot}107 \pm 0{\cdot}01$ ($n = 28$)		$3{\cdot}5 \pm 0{\cdot}4$ ($n = 27$)

* Rising phase of spike.
† Falling phase of spike.
‡ AH = after-hyperpolarization.

neurones responded to depolarizing current pulses by repetitive action potential discharge and a decreased amplitude of the after-hyperpolarization, coincident with a lower membrane resting potential and a higher input resistance. Similar findings were present upon impalement in eighteen AH/type 2 neurones. Neurones showing this behaviour tended to have a lower resting potential ($-59{\cdot}1 \pm 1{\cdot}7$ mV) than typical AH/type 2 neurones ($-63{\cdot}6 \pm 1{\cdot}6$ mV), but this difference was not statistically significant ($P = 0{\cdot}06$). The input resistance of 'activated' AH/type 2 neurones ($144{\cdot}9 \pm 19{\cdot}9$ MΩ) was significantly higher than in typical AH/type 2 neurones ($89{\cdot}2 \pm 11{\cdot}0$ MΩ, $P = 0{\cdot}02$). Anodal break excitation was observed in six of the 'activated' AH/type 2 neurones.

Action potentials

Action potentials in antral neurones were evoked experimentally by: (1) intracellular injection of depolarizing current pulses; (2) electrotonic invasion of the soma by inbound spikes in neurites triggered by focal electrical stimulation of

interganglionic fibre tracts; (3) excitatory synaptic potentials that were triggered by fibre tract stimulation (Tack & Wood, 1991). The amplitudes of the action potentials in gastric I, II and AH/type 2 neurones ranged from 60 to 105 mV, with positive overshoots of 5–15 mV. In gastric I and II cells, the rate of repolarization of the membrane during the falling phase of the action potential accelerated uniformly with time. In contrast, in AH/type 2 cells, a broadening of the falling phase of the action potential was present (Fig. 1). This was seen on records of dV/dt as a decreased slope. Duration of the spikes in AH/type 2 cells was significantly longer than in gastric I and II cells (Table 2).

In gastric I and II neurones, the action potentials were followed by a short after-hyperpolarization, continuous with the negative undershoot of the spike (Table 2). The short after-hyperpolarization did not summate when multiple spikes were fired. During the short hyperpolarizing after-potential, the input resistance was decreased, as revealed by the decreased amplitude of electrotonic potentials. In gastric I and gastric II neurones, the amplitude of the after-hyperpolarization was progressively decreased when the membrane potential was current clamped in steps to greater levels of hyperpolarization. The after-hyperpolarization disappeared at membrane potentials of about -90 mV, suggesting increased K^+ conductance as a mechanism.

In AH/type 2 neurones, the action potentials were associated with long-lasting hyperpolarizing after-potentials, which began 30–80 ms after the positive after-potential of the spike (Table 2). The amplitude of the long after-hyperpolarization in AH/type 2 neurones increased when an increasing number of action potentials was fired.

During the hyperpolarizing after-potential, the input resistance was decreased, as demonstrated by decreased amplitude of electrotonic potentials (Fig. 1). In AH/type 2 cells, the amplitude of the after-hyperpolarization gradually decreased when the cell was current clamped at progressively greater levels of hyperpolarization. At membrane potentials of about -90 mV, the after-hyperpolarization disappeared, suggesting increased K^+ conductance as a mechanism. Overall, the hyperpolarizing after-potentials were like those seen in intestinal AH/type 2 neurones (Nishi & North, 1973; Hirst, Holman & Spence, 1974; Grafe *et al.* 1980).

Effects of tetrodotoxin, tetraethylammonium, 4-aminopyridine, apamin and depleted Ca^{2+}

In three gastric I and seven gastric II neurones application of tetrodotoxin (0·1–5 μM) reversibly blocked the action potentials evoked by depolarizing current pulses (Fig. 2). This suggested exclusive involvement of inward Na^+ current in the depolarization phase of the spike. Addition of tetraethylammonium (5–20 mM), to block delayed rectifier K^+ current in the presence of tetrodotoxin, restored the spikes (Fig. 2). These spikes were broadened by a prolongation of the repolarization phase (Fig. 2*C*). This appeared as a region of decreased slope on dV/dt records, reminiscent of the 'shoulder' on the spikes of AH/type 2 neurones.

Addition of tetraethylammonium to the superfusion solution without tetrodotoxin also caused the appearance of a 'shoulder' on the falling phase of the spikes in gastric I and II neurones (Fig. 2). Coincident with the broadening of the action potential, tetraethylammonium also caused an increase in the amplitude of the action potential

(up to 15 %) and a broadening of the after-hyperpolarization. This probably reflects blockade by tetraethylammonium of the delayed rectifier K^+ channels that normally are responsible for the repolarization phase. Removal of Ca^{2+} from the bathing solution partially reversed the effects of tetraethylammonium. Removal of Ca^{2+} from

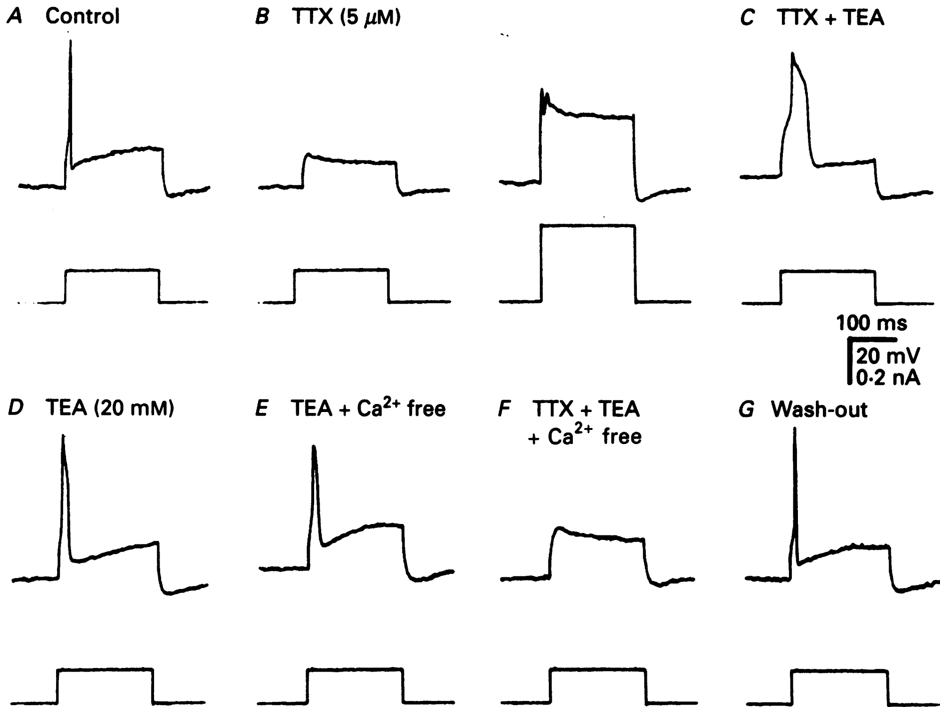

Fig. 2. Effects of tetrodotoxin (TTX), tetraethylammonium (TEA) and removal of Ca^{2+} on the electrophysiological behaviour of a gastric II neurone in the myenteric plexus of guinea-pig gastric antrum. *A*, the control response consisted of a single action potential at the onset of a depolarizing current pulse. *B*, application of tetrodotoxin (0·5 μM) blocked the spike discharge to pulses of control strength. Increase in the current strength evoked small oscillations at the onset of the depolarizing current pulse. *C*, addition of 20 mM-tetraethylammonium in the continued presence of tetrodotoxin restored the spike to a pulse of control amplitude. This spike was broadened with a pronounced 'shoulder' on the falling phase. *D*, addition of tetraethylammonium without tetrodotoxin also caused the appearance of a 'shoulder' on the falling phase of the spike. *E*, removal of Ca^{2+} from the bathing medium in the presence of tetraethylammonium suppressed the 'shoulder'. *F*, no spikes were evoked after removal of Ca^{2+} from the bathing medium containing tetrodotoxin and tetraethylammonium. *G*, return to control behaviour after wash-out in normal Krebs solution. Upper trace is transmembrane voltage; lower trace is injected current.

the bathing solution, in the presence of tetrodotoxin and tetraethylammonium, abolished all spikes (Fig. 2*F*). The spikes returned to normal 15–20 min after wash-out. Removal of Ca^{2+} from the bathing medium suppressed the hyperpolarization, while tetraethylammonium (5–15 mM) prolonged the hyperpolarization, coincident with broadening of the action potential (Fig. 3).

Treatment with the K^+ channel blocker, 4-aminopyridine (10 mM), reduced the amplitude and duration of the hyperpolarizing after-potential without affecting the shape of the action potential (Fig. 3*C*). Apamin (1 nM–1 μM), another putative K^+ channel blocker, did not affect the shape of the action potentials or the after-hyperpolarization in gastric I and II neurones (Fig. 3*E*).

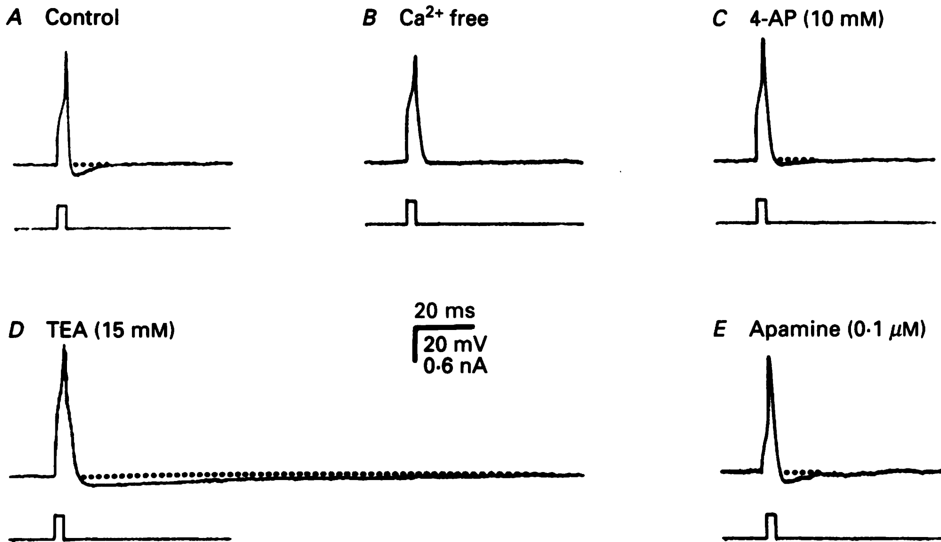

Fig. 3. Effects of tetraethylammonium, 4-aminopyridine, apamin and removal of Ca^{2+} on the hyperpolarizing after-potential in a gastric II neurone in the myenteric plexus of guinea-pig gastric antrum. *A*, control hyperpolarizing after-potential. *B*, removal of Ca^{2+} from the bathing medium suppressed the after-hyperpolarization. *C*, treatment with 4-aminopyridine decreased the amplitude and the duration of the after-hyperpolarization. *D*, tetraethylammonium prolonged the after-hyperpolarization. *E*, apamin did not affect the hyperpolarizing after-potential. Upper trace is transmembrane voltage; lower trace is injected current.

Both tetraethylammonium and 4-aminopyridine (10–20 mM) increased the excitability of gastric I and II neurones. This was apparent as a lowering of the spike threshold. At the highest concentrations, tetraethylammonium and 4-aminopyridine evoked a depolarization and the occurrence of spontaneous spike discharge. During tetraethylammonium, but not 4-aminopyridine, this occurred coincident with the appearance of hyperpolarizing after-potentials and broadening of the action potentials. The effects of tetraethylammonium and 4-aminopyridine were reversible after 10–15 min wash. These actions of the K^+ channel blockers were the same as in gastric I and II neurones of the gastric corpus (Schemann & Wood, 1989*a*). Like neurones of the corpus, resting K^+ conductance seems to suppress excitability.

In five AH/type 2 neurones, application of tetrodotoxin (0·1–5 μM) never abolished the action potentials, although the amplitude and the rate of rise of the spikes was decreased, and often the threshold for spike discharge was higher (Fig. 4). Superfusion of low-calcium–high-magnesium Krebs solution in the presence of tetrodotoxin

abolished all action potentials (Fig. 4*C*). This suggests that Ca^{2+} contributes to the inward current of the spike in these neurones.

Low-calcium–high-magnesium Krebs solution without tetrodotoxin suppressed the 'shoulder' on the falling phase of the action potential in all of four cells (Fig. 4*D*).

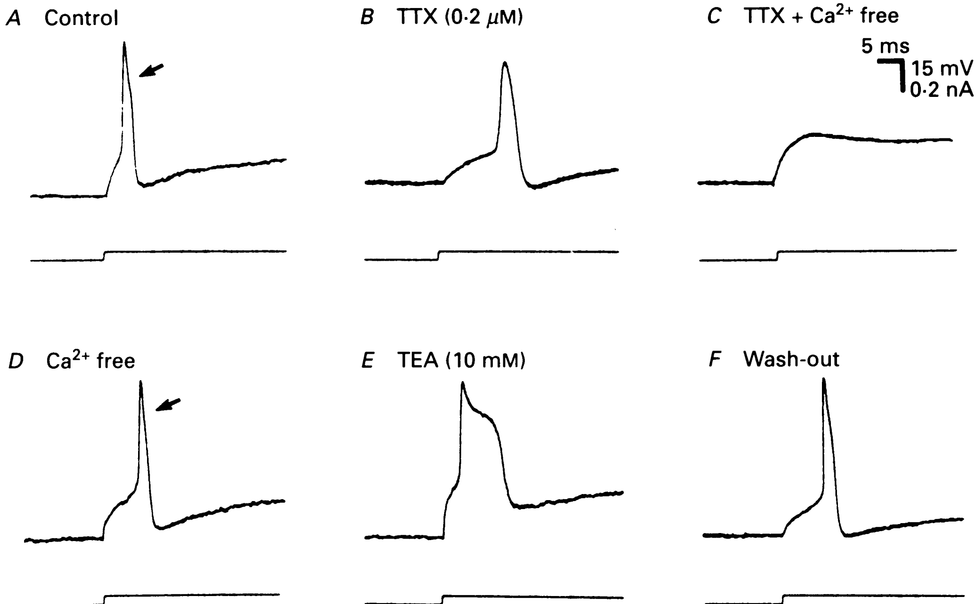

Fig. 4. Effects of tetrodotoxin, tetraethylammonium and removal of Ca^{2+} on the electrophysiological behaviour of an AH/type 2 neurone in the myenteric plexus of guinea-pig gastric antrum. *A*, control response consisted of an action potential at the onset of a depolarizing current pulse. *B*, application of tetrodotoxin failed to abolish the action potential. *C*, removal of Ca^{2+} in the presence of tetrodotoxin abolished all spike discharge. *D*, removal of Ca^{2+} without tetrodotoxin suppressed the 'shoulder' (arrows) on the falling phase of the action potential. *E*, application of tetraethylammonium broadened the 'shoulder'. *F*, return to control behaviour after wash-out in normal Krebs solution. Upper trace is transmembrane voltage; lower trace is injected current.

Suppression of the 'shoulders' on the action potentials in reduced Ca^{2+} suggests that the 'shoulders' result from opening of Ca^{2+} channels. Tetraethylammonium (5–10 mM) enhanced the after-hyperpolarization, coincident with a broadening of the action potential in all of five neurones (Fig. 4*E*). Superfusion of 4-aminopyridine (10 mM) also broadened the action potential, coincident with a decrease in amplitude and an increase of the duration of the after-hyperpolarization (Fig. 4*C*). Superfusion with normal Krebs solution for 10–15 min restored the action potential to normal. Apamin (1 nM–1 M) did not affect the shape of the action potential in three neurones. This component of bee venom, which prevents opening of K^+ channels in intestinal smooth muscle cells (Vladimirova & Shuba, 1978), appeared to have no effects on the neuronal K^+ channels.

Treatment with tetraethylammonium (5–10 mM) enhanced the amplitude and the duration of the after-hyperpolarization, coincident with broadening of the spike in AH/type 2 neurones (Fig. 5). Removal of Ca^{2+} from the superfusion solution

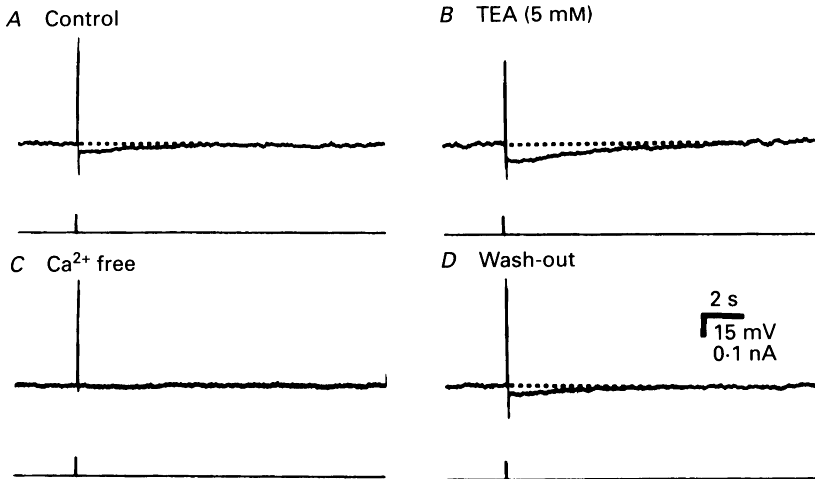

Fig. 5. Effects of tetraethylammonium and removal of Ca^{2+} on the after-hyperpolarization in an AH/type 2 neurone in the myenteric plexus of guinea-pig gastric antrum. *A*, control response. *B*, tetraethylammonium enhanced the amplitude and the duration of the after-hyperpolarization. *C*, removal of Ca^{2+} from the bathing solution abolished the after-hyperpolarization. Return to control behaviour after wash-out in normal Krebs solution. Upper trace is transmembrane voltage; lower trace is injected current.

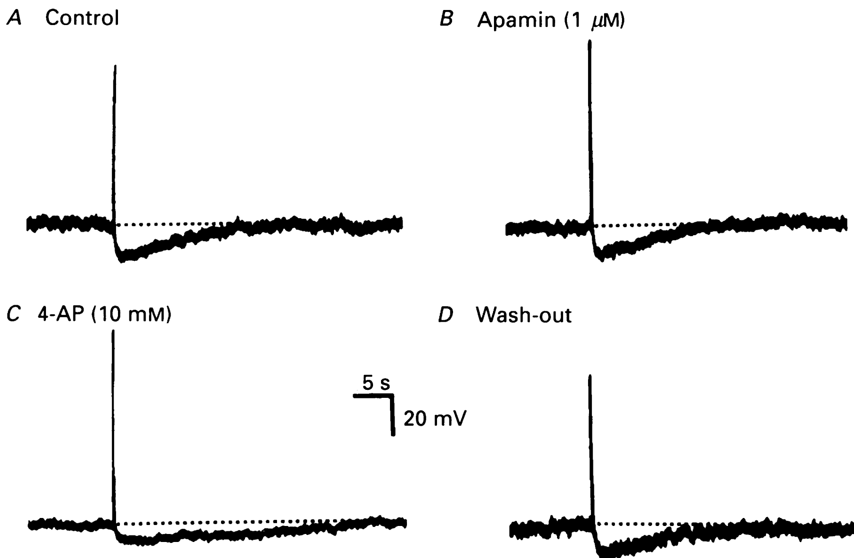

Fig. 6. Effects of apamin and 4-aminopyridine on the after-hyperpolarization in an AH/type 2 neurone in the myenteric plexus of the guinea-pig gastric antrum. *A*, control response. *B*, apamin did not affect the after-hyperpolarization. *C*, 4-aminopyridine decreased the amplitude and prolonged the duration of the after-hyperpolarization. *D*, return to control behaviour after wash-out in normal Krebs solution.

suppressed the amplitude and the duration of the after-hyperpolarization (Fig. 5*C*). This occurred in association with depolarization, enhanced excitability and increased input resistance, thus mimicking the 'activated' state of AH/type 2 neurones.

The results of the experiments with tetraethylammonium and depleted Ca^{2+} support earlier evidence that the hyperpolarizing after-potentials reflect opening of Ca^{2+}-dependent K^+ channels brought about by influx of Ca^{2+} during the action potential (North, 1973; Grafe *et al.* 1980; Hirst, Johnson & Van Helden, 1985*a*, *b*). The presence of a significant inward Ca^{2+} current during the spike in antral AH/type 2 neurones appears to be one of the factors underlying the differences in behaviour between these and the gastric I and III neurones. The effects of lowering external Ca^{2+} were like those in intestinal AH/type 2 neurones which are attributed to closure of Ca^{2+}-dependent K^+ channels (Grafe *et al.* 1980; Hirst *et al.* 1985*a*, *b*). Like AH/type 2 neurones in the small intestine, outward current in Ca^{2+}-dependent K^+ channels also appears to contribute to the resting potential in antral AH/type 2 neurones.

Application of 4-aminopyridine (10 mM) decreased the amplitude and increased the duration of the after-hyperpolarization (Fig. 6*C*). Apamin (1 nM-1 μM) did not affect the hyperpolarizing after-potential in AH/type 2 neurones (Fig. 6*B*).

Responses to forskolin

Forskolin is a substance that specifically activates adenylate cyclase. This makes it a useful tool for investigation of cellular responses that depend on cyclic adenosine 3′,5′-monophosphate (cyclic AMP) as a second messenger (Seamon, Padgett & Daly, 1980). Micropressure application of forskolin did not cause any changes in the membrane potential, the input resistance or the excitability of nine gastric I and twenty-seven gastric II neurones.

In five gastric III neurones, forskolin also had no effect, whereas in three gastric III neurones, it caused depolarization associated with an increase in the input resistance and enhanced excitability. After application of forskolin, these three neurones became excitable and behaved as AH/type 2 cells.

In twelve of thirteen cells classified as AH/type 2 neurones, micropressure application of forskolin caused depolarization of the membrane potential, an increase in the input resistance, reduction of the hyperpolarizing after-potentials and enhanced excitability (Fig. 7). This effect also was observed during the superfusion of forskolin (1 μM) and was reversed by 10–15 min of washing. All actions of forskolin were preserved during superfusion of 0·1 μM-tetrodotoxin, suggesting a direct action on the impaled neurone.

Behavioural plasticity of antral neurones

Sometimes, when the impalements were maintained for hours, interconversion from one behavioural type to another was seen. Changes from gastric II behaviour to gastric I behaviour were seen in twenty-one neurones, and fourteen gastric I neurones converted to a behavioural state characteristic for gastric II cells. Eleven gastric III cells converted to gastric II behaviour and six gastric III cells changed to AH/type 2 behaviour. One gastric II neurone and one AH/type 2 cell became inexcitable during the course of the experiment. Ten AH/type 2 neurones became 'activated' during the experiments, and two 'activated' AH/type 2 neurones reverted to a typical AH/type 2 behaviour.

Conversion from one type to another involved all of the relevant identifying

properties including membrane resisting potential, input resistance and level of excitability. Behavioural interconversions between AH/type 2 and gastric I or II cell types were not observed.

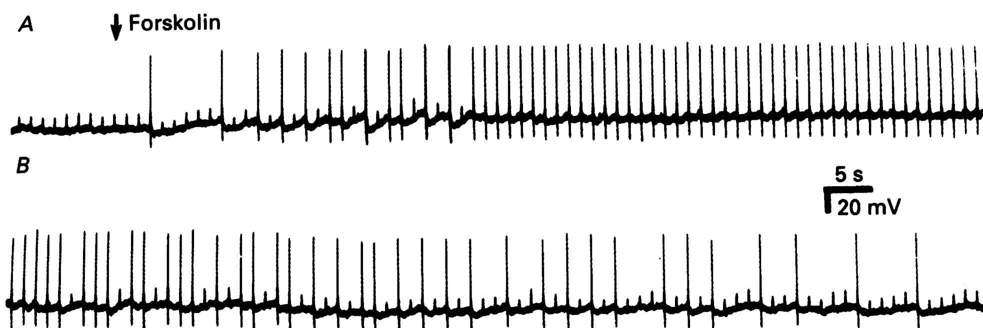

Fig. 7. Effects of forskolin on an AH/type 2 neurone in the myenteric plexus of the guinea-pig gastric antrum. *A*, micropressure application of a 15 ms pulse of 1 μM-forskolin caused a long-lasting depolarization with enhanced excitability and suppression of the hyperpolarizing after-potential. *B*, record starts 1 min after the end of trace *A*.

DISCUSSION

The first intracellular electrophysiological studies of enteric neurones were done in the myenteric plexus of the guinea-pig small intestine (Nishi & North, 1973; Hirst *et al.* 1974; Wood & Mayer, 1978). Based on their electrical behaviour, neurones were classified as S/type 1 neurones, AH/type 2 neurones and type 3 neurones. S/type 1 neurones were characterized by a low membrane potential, a high input resistance, repetitive discharge of tetrodotoxin-sensitive spikes and nicotinic cholinergic fast synaptic input (fast EPSPs). AH/type 2 neurones were characterized by a lower excitability, tetrodotoxin-resistant spikes with long-lasting hyperpolarizing after-potentials and apparent absence of synaptic input. Type 3 neurones were inexcitable, had a high membrane potential and low input resistance (see reviews by Wood, 1987*a*, *b*, 1989). The same classification was applicable for the myenteric plexus of the rectum (Tamura & Wood, 1989) and the submucous plexus of the small intestine (Hirst & McKirdy, 1975).

In the myenteric and submucous plexuses of the colon, an additional subpopulation of neurones, referred to as type 4 neurones, was described (Wade & Wood, 1988*a*; Frieling, Cooke & Wood, 1989). These neurones were characterized by low excitability evidenced by discharge of only one or two spikes during suprathreshold depolarizing current pulses. The spikes were tetrodotoxin sensitive and without long-lasting hyperpolarizing after-potentials.

A later study of electrophysiological behaviour of myenteric neurones in the gastric corpus revealed significantly different behaviour form that found in intestinal neurones (Schemann & Wood, 1989*a*). Three subpopulations of neurones were distinguished and classified as gastric I, II and III. Gastric I neurones were characterized by repetitive spike discharge during depolarizing current pulses and by high input resistances. Gastric II neurones discharged one or two spikes at the onset

of depolarizing current pulses. In both gastric I and II neurones, action potentials were suppressed by tetrodotoxin and not followed by a long-lasting after-hyperpolarization. Gastric III neurones did not discharge spikes to depolarizing current pulses and had higher membrane potentials and lower input resistances than the other types. No behaviour characteristic of AH/type 2 intestinal neurones was observed in the gastric corpus.

The electrical behaviour of myenteric ganglion cells in the gastric antrum had some similarities, but also considerable differences compared to the myenteric plexus of both the gastric corpus and the small and large intestine. Because the majority of the neurones behaved like the ganglion cells in the gastric corpus, we used the same classification criteria for the antrum. However, an additional subpopulation of neurones with distinctive electrical behaviour was present in the gastric antrum. We called these AH/type 2 neurones because they shared common characteristics with this type of neurone in the small intestine, colon and rectum.

Gastric I neurones

Gastric I neurones in the antrum were similar to S/type 1 neurones in the enteric nervous system of the small intestine, colon and rectum (Nishi & North, 1973; Hirst *et al.* 1974; Tamura & Wood, 1989; Frieling *et al.* 1989). However, unlike S/type 1 neurones elsewhere, the repetitive spike discharge did not continue during sustained depolarization and anodal break excitation was rarely seen.

The behaviour of antral gastric I neurones was the same as gastric I neurones in the corpus of the stomach (Schemann & Wood, 1989*a*), whereas the relative proportion of gastric I neurones to the total number of neurones sampled was considerably lower than in the gastric corpus. This may reflect adaptations for the different functions of the corpus and the antrum.

Gastric II neurones

Gastric II neurones were comparable to intestinal and rectal AH/type 2 neurones. Nevertheless, unlike AH/type 2 cells, the action potentials were blocked by tetrodotoxin and they were not associated with a long-lasting hyperpolarizing after-potential. The properties of gastric II neurones are reminiscent of the small intestinal myenteric neurones described by Hodgkiss & Lees (1978, 1983) that could not be categorized as either S/type 1 or AH/type 2 neurones. They are also similar to the type 4 neurones found in the colonic myenteric and submucous plexuses of the guinea-pig (Wade & Wood, 1988*a*; Frieling *et al.* 1989).

The characteristics of gastric II neurones in the gastric antrum were the same as those described for gastric II neurones in the gastric corpus (Schemann & Wood, 1989*a*). The ratio of gastric II neurones to the total of the sampled population was higher in the antrum than in the corpus (Schemann & Wood, 1989*a*).

Gastric III neurones

Gastric III neurones in the antrum did not discharge action potentials in response to high-amplitude depolarizing current pulses. Based on the presence of nicotinic cholinergic synaptic input and neurite spikes, they could be identified as neurones (Tack & Wood, 1991). Gastric III neurones had a resting membrane potential higher

than in other cell types, whereas the membrane time constant and the input resistance were lower than in the other cell types. These characteristics probably reflect a high K^+ conductance in the resting state. In these respects, gastric III neurones behaved like small intestinal, colonic and rectal type 3 cells (Wood, 1987*b*, 1989; Wade & Wood, 1988*a*; Frieling *et al.* 1989; Tamura & Wood, 1989) and like the gastric III neurones in the corpus of the stomach (Schemann & Wood, 1989*a*).

Enteric neurones with behaviour like the gastric III cells have been interpreted as 'dormant' neurones in which the spike generation mechanism is inactive until the binding of a specific excitatory messenger to receptors on the somal membrane stimulates excitability (Wood, 1987*a*, *b*). Type 3 neurones in the small intestine were inexcitable until activated to discharge spikes by receptor-mediated stimulation of adenylate cyclase (Palmer, Wood & Zafirov, 1987). In the present study, we observed elevation of cyclic AMP by forskolin to be an excitatory factor in a subgroup of the antral gastric III neurones. These cells converted to AH/type 2 behaviour after activation.

A lower proportion of inexcitable cells was observed in the antrum than was reported for the gastric corpus (Schemann & Wood, 1989*a*). This may also be an indication of specialization of the microcircuits in these functionally distinct regions.

AH/type 2 neurones

AH/type 2 neurones in the antrum had characteristics similar to the AH/type 2 neurones described in the small intestine, the colon and the rectum of the guinea-pig (Nishi & North, 1973; Hirst *et al.* 1974; Wade & Wood, 1988*a*; Frieling *et al.* 1989; Tamura & Wood, 1989). AH/type 2 behaviour was not found in the myenteric plexus of the gastric corpus (Schemann & Wood, 1989*a*).

The presence of AH/type 2 neurones in the antral myenteric plexus may be related to the functional specialization within the stomach. Propulsive motility in the antrum, undoubtedly, requires a set of microcircuits different from those that program the tonic contractions and receptive relaxation functions found in the corpus. The presence of AH/type 2 neurones in the antrum and their absence from the corpus probably reflect adaptations of the microcircuits for organization of the specific motor patterns found in each region. In general, AH/type 2 neurones are found in regions of the digestive tract where peristaltic propulsion is a prominent functional event.

The results obtained with forskolin in AH/type 2 neurones suggest involvement of cyclic AMP as a second messenger in excitatory signal transduction in these neurones, but not in the gastric I and II types. This is consistent with observations in the small intestine where application of forskolin or membrane-permeable analogues of cyclic AMP mimic slow synaptic excitation in AH/type 2 neurones, but not S/type 1 cells (Nemeth, Palmer, Wood & Zafirov, 1986; Palmer, Wood & Zafirov, 1986). Excitatory neuromodulation mediated by receptor activation of adenylate cyclase appears to occur only in enteric microcircuits in which AH/type 2 neurones make up some of the interconnected neural elements. Involvement of AH/type 2 neurones and the accompanying phenomenon of slow synaptic excitation in the antrum and not the corpus is another indication of functional specialization in the two divisions of the stomach.

In the myenteric plexus of the small intestine, inexcitable cells comparable to antral gastric III neurones are activated by forskolin to behave like AH/type 2 neurones (Nemeth *et al.* 1986). The low-excitability state of these cells is related to low intraneuronal levels of cyclic AMP. Stimulation of adenylate cyclase and elevation of cyclic AMP convert the neurone to an active state. Gastric III cells in the antrum behaved in a similar manner suggesting that they are the behavioural analogues of the inexcitable cells found in the intestine.

Behavioural plasticity

The patterns of behaviour for the types of neurones in the antrum were not permanently fixed. Conversion between gastric I and gastric II patterns was observed during long-lasting impalements. This suggests that gastric I and II behaviour reflects different activation states of a single type of neurone. Conversion between gastric II and gastric III behaviour was also observed, suggesting that some gastric III neurones were gastric I and II neurones which had converted to a 'dormant' state. However, not all neurones with gastric III behaviour could be considered 'dormant' gastric I or II neurones, because some of the population displayed AH/type 2 behaviour after activation by forskolin. This is evidence that some of the population of gastric III neurones were 'silent' AH/type 2 neurones. Further plasticity of behaviour of AH/type 2 neurones was evident in conversion from typical AH/type 2 behaviour to an activated state with repetitive spike discharge, increased input resistance and suppression of the hyperpolarizing after-potentials. No interconversion between AH/type 2 and gastric I or II behaviour was ever observed.

The findings are consistent with the existence of only two fundamentally different cell populations within the myenteric plexus of the gastric antrum. Impressions of additional types of neurones reflect plasticity of behaviour derived from neural or paracrine modulatory influences within the two populations. One of these populations exhibits behaviour that can interchange from inexcitability to gastric I to gastric II behaviour. These neurones are not activated by forskolin and have purely Na^+ spikes without long-lasting hyperpolarizing after-potentials. The behaviour of the second population can range from inexcitability to AH/type 2 behaviour to a state of enhanced excitability. These neurones are activated by forskolin, have tetrodotoxin-resistant Ca^{2+} spikes and in the typical AH/type 2 state have long-lasting hyperpolarizing after-potentials.

Plasticity of electrophysiological behaviour appears to be a fundamental property of enteric neurones whether in the gastric antrum or other regions of the digestive tract (Hodgkiss & Lees, 1978, 1983; Tamura & Wood, 1989). The behavioural states of the neurones are probably determined by changes in the concentrations of neurotransmitters and hormones in the external milieu. This in turn determines the dynamic state of the microcircuits which organize the patterns of behaviour seen in the musculature and mucosal epithelium of the functioning gut.

This work was supported by National Institutes of Health grant R01 DK37238 to J.D.W. and a grant from the Nationaal Fonds voor Wetenschappelijk Onderzoek, Belgium to J.T.

REFERENCES

ALLESCHER, H. D., DANIEL, E. E., DENT, J., FOX, J. E. T. & KOSTOLANSKA, F. (1988). Extrinsic and intrinsic neural control of pyloric sphincter pressure in the dog. *Journal of Physiology* **401**, 17–38.

BROOKES, S. J. H., EWART, W. R. & WINGATE, D. L. (1987). Intracellular recordings from myenteric neurones in the human colon. *Journal of Physiology* **390**, 305–318.

DELOOF, S. & ROUSSEAU, J. P. (1985). Neural control of electrical gastric activity in response to inflation of the antrum in the rabbit. *Journal of Physiology* **367**, 13–25.

FRIELING, T., COOKE, H. J. & WOOD, J. D. (1989). Electrophysiological properties of neurones in submucosal ganglia of the guinea-pig distal colon. *American Journal of Physiology* (in the Press).

FURUKAWA, K., TAYLOR, G. S. & BYWATER, A. R. (1986). An intracellular study of myenteric neurones in the mouse colon. *Journal of Neurophysiology* **55**, 1395–1406.

GERSHON, M. D. & ERDE, S. M. (1981). The nervous system of the gut. *Gastroenterology* **80**, 1571–1594.

GRAFE, P., MAYER, C. J. & WOOD, J. D. (1980). Synaptic modulation of calcium-dependent potassium conductance in myenteric neurones in the guinea-pig. *Journal of Physiology* **305**, 235–248.

HIRST, G. D. S., HOLMAN, M. E. & SPENCE, I. (1974). Two types of neurones in the myenteric plexus of the duodenum in the guinea-pig. *Journal of Physiology* **236**, 303–326.

HIRST, G. D. S., JOHNSON, S. M. & VAN HELDEN, D. F. (1985*a*). The calcium current in a myenteric neurone of the guinea-pig ileum. *Journal of Physiology* **361**, 277–314.

HIRST, G. D. S., JOHNSON, S. M. & VAN HELDEN, D. F. (1985*b*). The slow calcium-dependent potassium current in a myenteric neurone of the guinea-pig ileum. *Journal of Physiology* **361**, 315–337.

HIRST, G. D. S. & MCKIRDY, H. C. (1975). Synaptic potentials recorded from neurones of the submucous plexus of guinea-pig small intestine. *Journal of Physiology* **249**, 369–385.

HODGKISS, J. P. & LEES, G. M. (1978). Correlated electro-physiological and morphological characteristics of myenteric neurones. *Journal of Physiology* **285**, 19–20*P*.

HODGKISS, J. P. & LEES, G. M. (1983). Morphological studies of electrophysiologically-identified myenteric plexus neurones of the guinea-pig ileum. *Neuroscience* **8**, 593–608.

NEMETH, P. R., PALMER, J. M., WOOD, J. D. & ZAFIROV, D. H. (1986). Effects of forskolin on electrical behavior of myenteric neurones in guinea-pig small intestine. *Journal of Physiology* **376**, 439–450.

NISHI, S. & NORTH, R. A. (1973). Intracellular recording from the myenteric plexus of the guinea-pig ileum. *Journal of Physiology* **231**, 471–491.

NORTH, R. A. (1973). The calcium-dependent slow after-hyperpolarization in myenteric plexus neurones with tetrodotoxin-resistant action potentials. *British Journal of Pharmacology* **49**, 709–711.

NORTH, R. A. (1986). Mechanisms of autonomic integration. In *Handbook of Physiology*, section 1, vol. IV, ed. BLOOM, F. E., pp. 115–153. American Physiological Society, Bethesda, MD, USA.

PALMER, J. M., WOOD, J. D. & ZAFIROV, D. H. (1986). Elevation of cyclic adenosine monophosphate mimics slow synaptic excitation in myenteric neurones of the guinea-pig. *Journal of Physiology* **376**, 451–460.

PALMER, J. W., WOOD, J. D. & ZAFIROV, D. H. (1987). Transduction of aminergic and peptidergic signals in enteric neurones of the guinea-pig. *Journal of Physiology* **387**, 371–383.

SCHEMANN, M. & WOOD, J. D. (1989*a*). Electrical behavior of myenteric neurones in the gastric corpus of the guinea-pig. *Journal of Physiology* **417**, 501–518.

SCHEMANN, M. & WOOD, J. D. (1989*b*). Synaptic behavior of myenteric neurones in the gastric corpus of the guinea-pig. *Journal of Physiology* **417**, 519–535.

SEAMON, K. B., PADGETT, W. & DALY, J. W. (1980). Forskolin: unique deterpene activator of adenylate cyclase in membranes and in intact cells. *Proceedings of the National Academy Sciences of the USA* **78**, 3363–3367.

TACK, J. F. & WOOD, J. D. (1990). Electrical behavior of myenteric ganglion cells in the gastric antrum of the guinea-pig. *Gastroenterology* **98**, A396.

TACK, J. F. & WOOD, J. D. (1991). Synaptic behavior in the myenteric plexus of the guinea-pig gastric antrum. *Journal of Physiology* **445**, 389–406

TAMURA, K. & WOOD, J. D. (1989). Electrical and synaptic properties of myenteric plexus neurones in the terminal large intestine of the guinea-pig. *Journal of Physiology* **415**, 275–298.

VLADIMIROVA, I. A. & SHUBA, M. F. (1978). Effects of strychnine, hydrastine, and apamin on synaptic transmission in smooth muscle. *Neurophysiology* **78**, 213–217.

WADE, P. R. & WOOD, J. D. (1988*a*). Electrical behavior of myenteric neurones in guinea pig distal colon. *American Journal of Physiology* **254**, G522–530.

WADE, P. R. & WOOD, J. D. (1988*b*). Synaptic behavior of myenteric neurones in guinea pig distal colon. *American Journal of Physiology* **255**, G184–190.

WOOD, J. D. (1987*a*). Neurophysiological theory of intestinal motility. *Japan Journal of Smooth Muscle Research* **23**, 143–186.

WOOD, J. D. (1987*b*). Physiology of the enteric nervous system. In *Physiology of the Gastrointestinal Tract*, 2nd edn, ed. JOHNSON, L. R., pp. 367–398. Raven Press, New York.

WOOD, J. D. (1989). Electrical and synaptic behaviour of enteric neurones. In *Handbook of Physiology, The Gastrointestinal System Motility and Circulation*, section 6, vol. I, ed. WOOD, J. D., pp. 465–517. Oxford University Press, New York.

WOOD, J. D. & MAYER, C. J. (1978). Intracellular study of electrical activity of auerbach's plexus in guinea-pig small intestine. *Pflügers Archiv* **374**, 265–275.