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MODULATION OF VAGAL EFFERENT FIBRE DISCHARGE BY MECHANORECEPTORS IN THE STOMACH, DUODENUM AND COLON OF THE FERRET

BY D. GRUNDY, A. A. SALIH AND T. SCRATCHERD
From the Department of Physiology, University of Sheffield, Sheffield S10 2TN

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SUMMARY

1. A single-fibre dissection technique was used to investigate the reflex modulation of vagal efferent fibre discharge by afferent fibres from various parts of the gastrointestinal tract of the urethane-anaesthetized ferret.

2. All but four of the 168 efferent fibres isolated in this study were spontaneously active. The majority of these had discharge frequencies of less than 6 spikes/sec.

3. All the efferent units received an afferent input from mechanoreceptors in the stomach. Two main types of response to gastric distension were seen: (i) an increase in efferent discharge and (ii) a decrease or complete suppression of efferent discharge.

4. The vagal efferent discharge was also modulated by duodenal and colonic distension, with the major effect being one of inhibition.

5. Bilateral vagotomy completely abolished the response to gastric distension in 68% of the units tested. The response to colonic and duodenal distension, however, was relatively unaffected by vagotomy. Thus the vagus provides the major afferent pathway from the stomach to these vagal efferent fibres, whilst the major input from the duodenum and colon is via a non-vagal pathway. Both vagal and splanchnic afferents therefore converge on to the vagal nucleus.

6. The destinations of these vagal efferent fibres and their possible functions are discussed.

INTRODUCTION

Vagal afferent fibres arising from the gastrointestinal tract outnumber vagal efferent fibres by approximately nine to one (Agostini, Chinnock, Daly & Murray, 1957). It is likely therefore that convergence of vagal afferent information onto efferent neurones in the dorsal motor vagal nucleus must be considerable. In the rat, single vagal efferent fibres have been shown electrophysiologically to receive an input from vagal afferent fibres originating from 'in-series' tension receptors over a large area of stomach (Davison & Grundy, 1978). Individual tension receptors, however, have only small receptive fields (Clarke, 1977). These gastric tension receptors, which respond to both distension and active contraction of the viscera, provide the afferent limb of vago–vagal reflexes which serve to stimulate gastric motility (Andrews, Grundy & Scratcherd, 1980*a*), to relax the stomach to accommodate the food entering

(Abrahamsson & Jansson, 1969, 1973) and to stimulate various gastrointestinal glands to secrete.

Gastrointestinal distension also stimulates splanchnic afferents (Morrison, 1977) and splanchnic reflexes elicited by distension have been shown to have powerful inhibitory influences on gastrointestinal motility (Jansson, 1969; Abrahamsson, 1974).

The purpose of the present study was therefore to investigate the convergence of afferent information on the discharge of vagal efferent fibres to include inputs from the duodenum and colon and to determine the relative contributions of vagal and splanchnic afferents in the reflexes elicited by visceral distension. A preliminary report of this work has been presented to the Physiological Society (Salih, 1980).

METHODS

Animals and operative procedures

The experiments were performed on male and female ferrets anaesthetized with a single intraperitoneal (I.P.) dose of urethane (1·5 g/kg). They were fed on a standard carnivore diet with free access to water, but were deprived of food for 18–24 hr before experimentation. A clear airway was maintained by intubating the trachea through the mouth. The stomach was intubated via the mouth and oesophagus and also from the duodenum through the pylorus. Inflation and deflation of the stomach was carried out through the oesophageal tube and intragastric pressure measured through the fluid-filled pyloric tube attached to a pressure transducer (SEM 480). A loop of duodenum was prepared by intubating the duodenum in an aboral direction from immediately below the pylorus and in an oral direction from an incision made 15 cm below the pylorus. A similar loop of colon was prepared with the most distal cannula introduced 5 cm above the anus. The rectal temperature was maintained between 38·5 and 39·5 °C on a homeostatic blanket. The right cervical vagus was exposed and contained in a paraffin pool. The nerve was carefully dissected away from the adjacent carotid artery and placed over a black Perspex tray. Single efferent nerve fibres were teased from the nerve trunk as previously described by Davison & Grundy (1978) for the rat. The activity in each strand was observed and those with respiratory and cardiovascular rhythms discarded. Vagotomy was performed either in the neck caudal to the recording electrodes or in the thorax. Following the latter, the animals were maintained by artificial ventilation.

Neurophysiological recording

Action potentials were recorded on conventional neurophysiological equipment with neural activity and intragastric pressure displayed on a Medelec M-scope (Medelec Limited). The integrated output of the spike discharge was fed from a Digitimer D130 to one channel of a flat-bed pen recorder, with gastric pressure recorded on the second channel (Bryans 2800).

METHODS

Altogether 168 single vagal efferent fibres were isolated in the present study. When the stomach was in the deflated state, all except four of the efferent units showed spontaneous activity, firing continuously with a low frequency irregular discharge. Ninety-five per cent of the units had spontaneous firing frequencies of 6 spikes/sec and of these, 63 % were below 2 spikes/sec. All the units were characterized by their response to gastric inflation. Of the 168 units, sixty were examined in detail for their response to duodenal inflation and of these forty-three units were also examined for their response to colonic inflation. The effect of vagotomy on the efferent responses to gastric duodenal and colonic inflation was also investigated.

Discharge patterns in efferent vagal nerves in response to gastric inflation

Vagal efferent units were either excited (54·8%) or inhibited (43·4%) by gastric distension (Fig. 1), although a small proportion (1·8%) showed a mixed response with inhibition of efferent discharge at low levels of gastric distension and excitation at higher levels. The threshold gastric volume required to elicit a response in the vagal

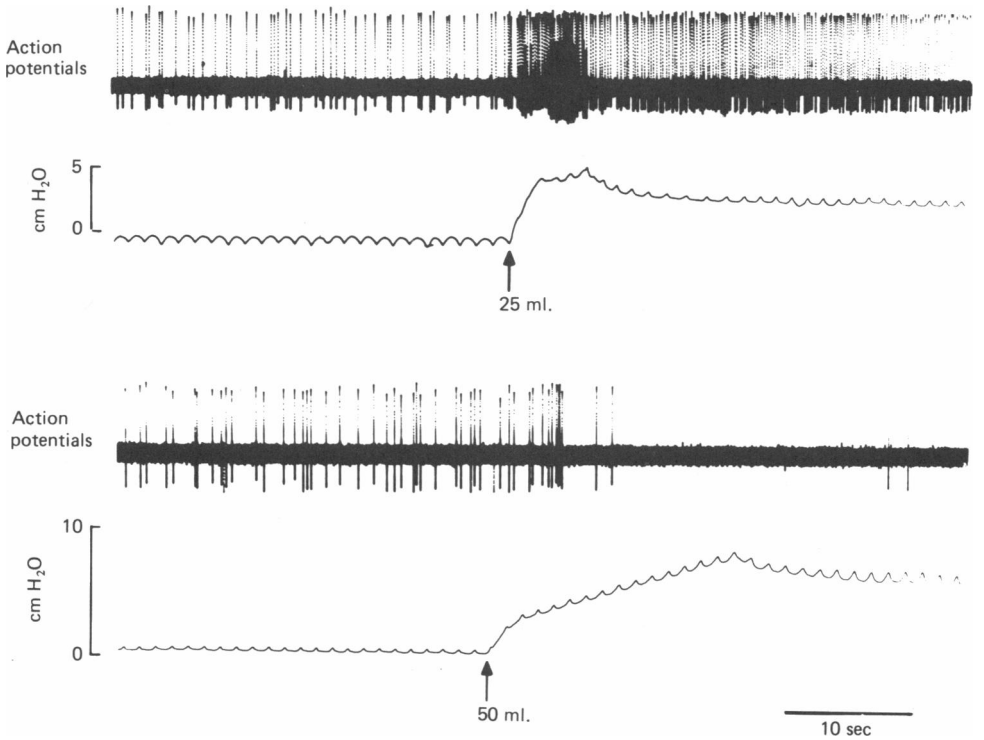

Fig. 1. Responses of two efferent units to gastric distension. In both records the upper trace shows the action potentials recorded from the nerve filament and the lower trace the intragastric pressure. The start of a gastric inflation is shown by the arrows. The figures below the arrows refer to the final intragastric volume. Note that in the upper record there is an increase in efferent discharge following gastric inflation and a transient recruitment of a second fibre, whilst in the lower record the efferent discharge is inhibited.

efferents varied from unit to unit but could be as low as 5 ml. ($< 0{\cdot}5$ cm H_2O) or as high as 75 ml. (15 cm H_2O). The following characteristics were noted. Once the threshold of gastric volume had been reached, the degree of excitation or inhibition in an individual unit was proportional to the level of inflation (Fig. 2). The efferent discharge was slowly or non-adapting to maintained inflations. However, the discharge could also be modulated by gastric motility. In those fibres which were excited by distension there was an increase in discharge coincidentally with antral contractions, whereas in those fibres which were inhibited the discharge was reduced when antral contractions occurred, provided the inhibition had not been total (Fig. 3). On the other hand some units showed no modulation with antral motility even when the propagated waves of contraction were quite pronounced.

An interesting feature of some of the efferent units was the response to deflation of the stomach. The discharge of the majority of units rapidly returned to pre-distension frequencies following deflation of the stomach (see Fig. 3), while others remained at the level of discharge attained during gastric distension for several minutes. An extreme example of this is one particular unit which maintained its elevated discharge

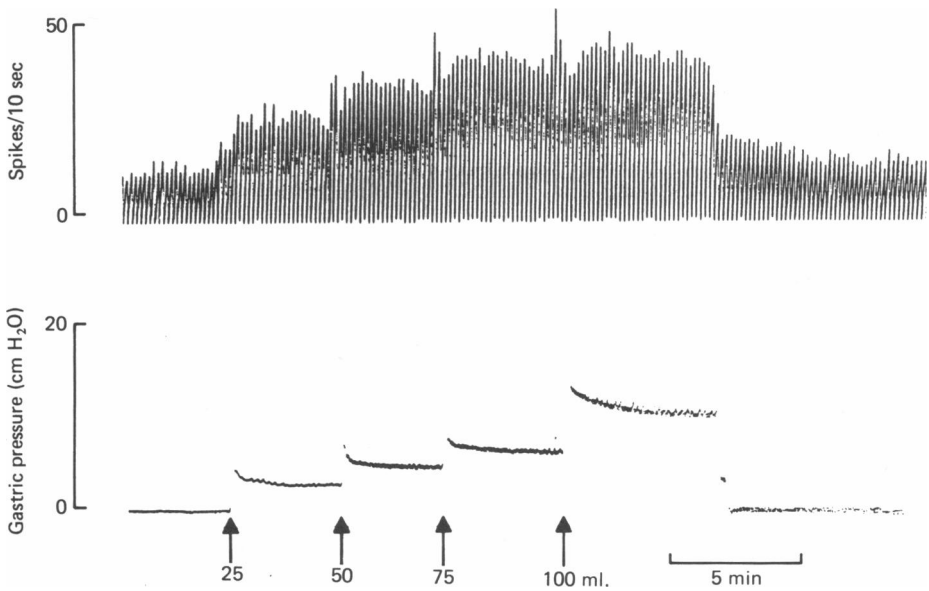

Fig. 2. Response of an efferent unit to step inflations of the stomach. Upper trace is an integrated record from a single vagal efferent fibre and represents the number of spikes in consecutive 10 sec periods; lower trace is the intragastric pressure. 25 ml. inflations of the stomach are shown by the arrows with the intragastric volume shown below. Note the increase in efferent discharge with increasing gastric volumes.

frequency for approximately 40 min following deflation (Fig. 4). The significance of these units will be discussed later.

The effect of vagotomy was investigated in twenty-five efferent units which were either excited or inhibited by gastric distension. The vagotomy was performed either in the neck, caudal to the recording electrodes (nine units) or in the thorax (sixteen units). In all cases, vagotomy was performed in two stages. First one vagal trunk was served and the effect on spontaneous efferent discharge and the response to gastric distension noted and then the other trunk was severed and the effect again noted. The response to gastric distension of 68% of the efferent units tested was completely abolished by bilateral vagotomy (Fig. 5). In these units, the degree to which the response was reduced by sectioning of one vagus nerve varied from unit to unit. Some efferent units showed no or very little reduction in their response to gastric inflation, while in others the response was greatly reduced. Sectioning the remaining vagal trunk in these units completely abolished the response to gastric inflation. Spontaneous discharge of these efferent units was also modified by vagotomy. In general, the efferent units inhibited by gastric distension showed an increased spontaneous discharge following vagotomy while the discharge frequency

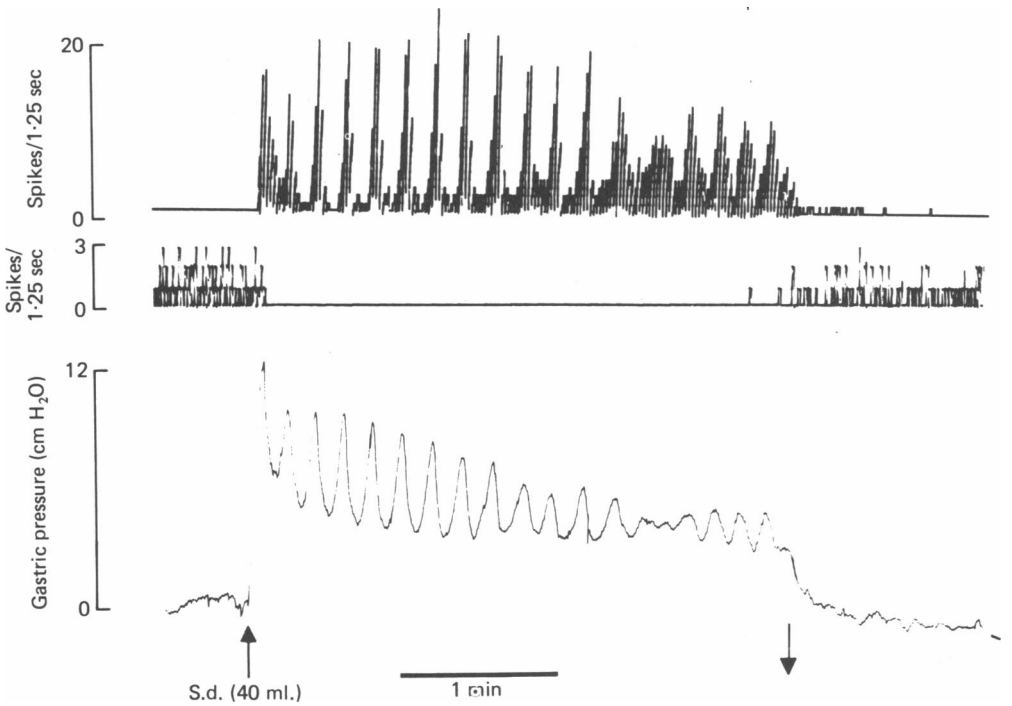

Fig. 3. Response of two vagal efferent units recorded from the same nerve filament to a 40 ml. step inflation of the stomach (s.d.). The amplitudes of the action potentials were sufficiently different to be discriminated electrically. Upper and middle trace: integrated record of spike discharge (reset time 1·25 sec); lower trace: intragastric pressure. Note that the upper unit shows marked modulation in phase with the antral contractions, whilst the lower unit is completely inhibited by gastric inflation.

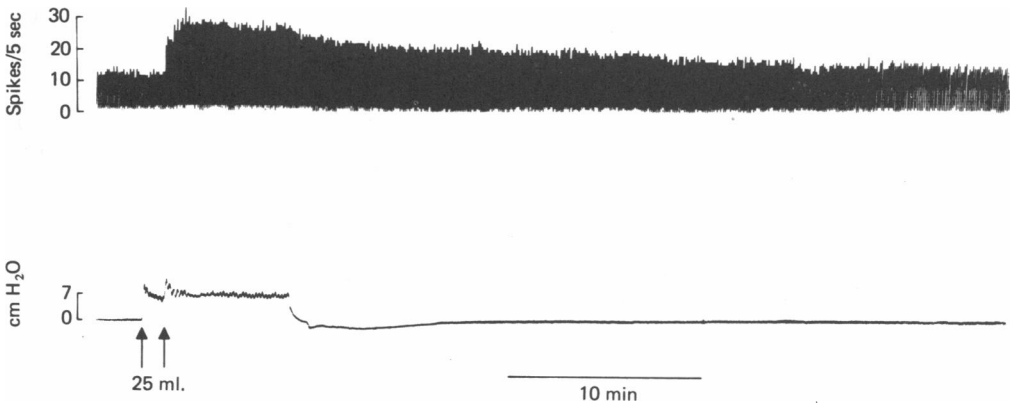

Fig. 4. Response of a vagal efferent unit to gastric distension. Upper trace: integrated spike discharge (reset time 5 sec); lower trace: intragastric pressure. This unit failed to respond to a 25 ml. inflation but a further 25 ml. caused an increase in efferent discharge. Note the slow return to the pre-distension rate of discharge following deflation of the stomach.

of efferent units excited by gastric inflation was reduced, and in four units completely abolished. Of the efferent units whose response to gastric distension was not completely abolished by total vagotomy, 16% were reduced, 8% were unaffected and 8% were reversed. Of the latter, one unit which was originally excited by gastric distension responded with inhibition of efferent discharge after vagotomy, and the

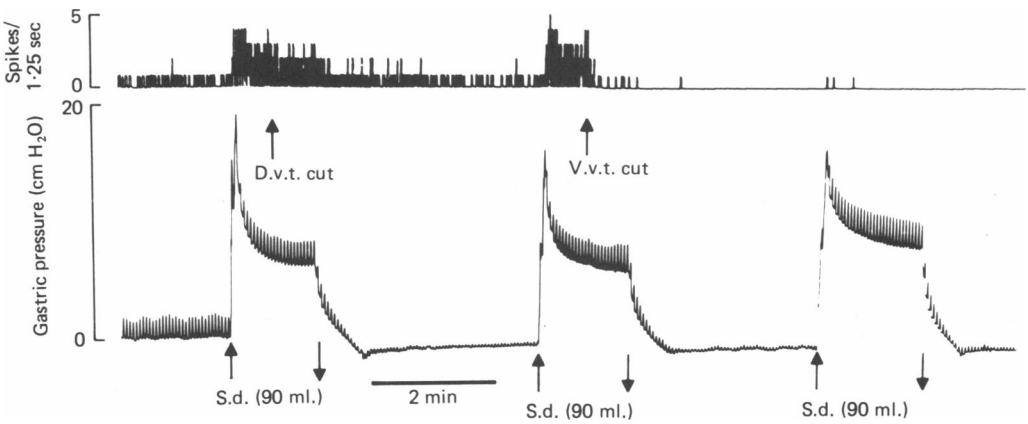

Fig. 5. Effect of vagotomy on a vagal efferent unit excited by gastric distension. Upper trace: integrated spike discharge (reset time 1·25 sec); lower trace: intragastric pressure. Stomach distension (s.d.) with 90 ml. is shown by the arrows. D.v.t.: dorsal vagal trunk. V.v.t.: ventral vagal trunk. Note that following bilateral vagotomy the spontaneous efferent discharge and the response to stomach distension are markedly reduced.

other, which was originally inhibited by gastric distension, was excited after vagotomy.

The influence of duodenal and colonic distension on vagal efferent discharge

These experiments were designed to test whether or not individual efferent vagal fibres could be driven from more than one site in the gastrointestinal tract. For this purpose two additional sites were chosen: the upper part of the small intestine, or the duodenum and the lower part of the intestine, designated here as colon. Once the efferent units response to gastric inflation had been established, the effect of duodenal and colonic distension with up to 8 ml. 0·9% NaCl was investigated. Although intraduodenal and intracolonic pressures were not routinely monitored, inflations with 8 ml. produced an intraluminal pressure of the order of 20 cm H_2O.

Duodenal distension

Of sixty efferent units tested with duodenal distension, fifty-two units showed a response, with the major effect being one of inhibition. The threshold volume for a response varied between units, it would be as low as 1 ml. but varied up to 5 ml. Duodenal distension inhibited either the spontaneous efferent discharge or the efferent discharge evoked by gastric distension. The efferent response to duodenal distension could be either slowly adapting, as with the response to gastric inflation and return to pre-distension levels following deflation, or rapidly adapting with the maximal effect occurring during the dynamic phase of the inflation. Although the

major effect was one of inhibition, four units were excited by duodenal distension and these were also excited by gastric distension. However, the majority of units excited by gastric distension were inhibited by duodenal distension and all units inhibited by gastric distension were also inhibited from the duodenum (Fig. 6). Vagotomy had very little effect on the efferent response to duodenal distension. Of

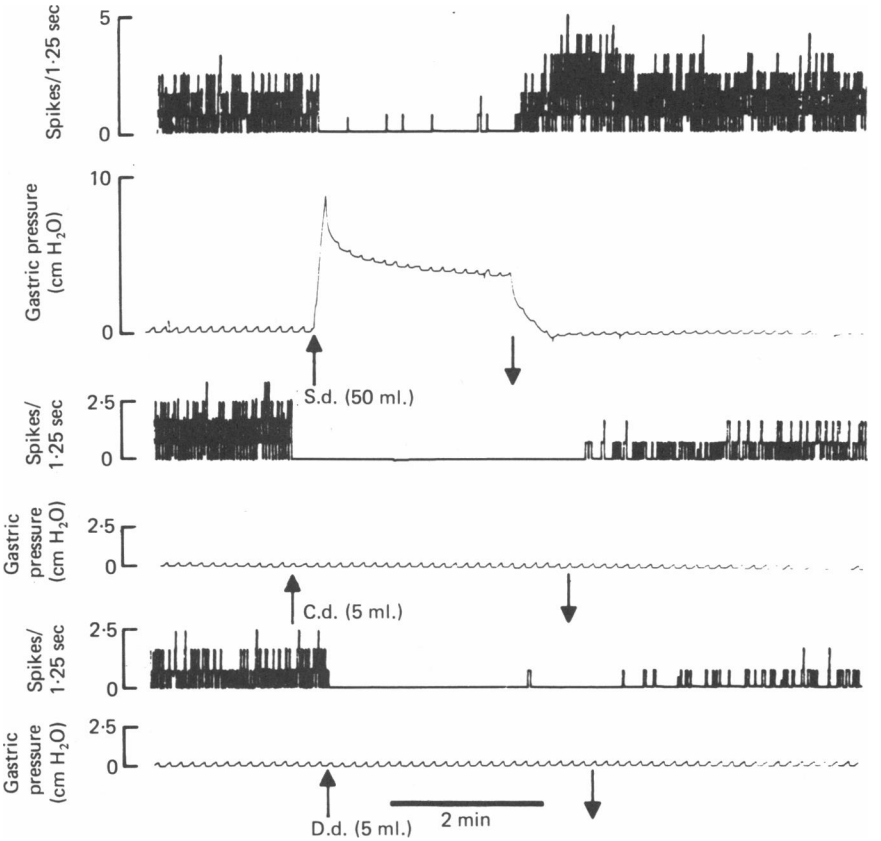

Fig. 6. Response of a single vagal efferent unit to stomach distension (s.d.): colonic distension (c.d.) and duodenal distension (d.d.). In each case, the upper trace is the integrated spike discharge (reset time 1·25 sec) and the lower trace the intragastric pressure. The duration of the distension is shown by the arrows.

the fourteen units tested, twelve were unaffected, one showed a reduced response and the other unit's response reversed from one of excitation to one of inhibition.

Colonic distension

The vagal efferent responses to colonic distension were similar to those for duodenal distension. Again the response was predominantly inhibition of efferent discharge (Fig. 6). When excitation did occur, this was only in units which were also excited by gastric distension. The threshold volume required to elicit the responses varied from unit to unit. Again vagotomy had very little effect on the efferent response to colonic distension. Of the fifteen units tested, twelve were unaffected and three showed a reduced response. The efferent units (forty-three) tested for both duodenal

and colonic inputs generally showed the same response for both, i.e. all the units inhibited by duodenal distension were also inhibited by colonic distension. The units excited by duodenal distension however, showed more variablity. Of the four units excited by gastric and duodenal distension, two were excited by colonic distension and two inhibited. One further unit was excited by gastric and colonic distension and unaffected by duodenal distension.

DISCUSSION

Afferent information from the stomach projects centrally in both vagi and modulates the vagal efferent discharge of 68 % of the units. Of the remaining efferent units, 16 % have a partial vagal input as shown by the reduced response to gastric inflation following vagotomy and a further 8 % have no vagal input. These latter units and the efferent units whose response changes from one type to another after vagotomy must have an afferent input from a non-vagal source. One possible afferent pathway would be the vagal branches which Harper, McSwiney & Suffolk (1935) suggested travel along with the intercostal nerves. However, because of the number involved and the observation that the afferent input from the duodenum and colon is also non-vagal, then this possibility seems unlikely. A more obvious pathway would be along the splanchnic nerves which have been shown to be activated by gastrointestinal distension (Morrison, 1977) and furthermore, the threshold for activation of these afferent fibres is comparable to vagal afferent fibres. Thus, splanchnic as well as vagal afferent fibres seem to impinge on the dorsal motor nucleus of the vagus thereby constituting a splancho–vagal reflex which has not been previously described.

With regard to gastric distension, since the majority of fibres receive only a vagal input, it is not surprising that the efferent response to gastric distension can be adequately explained on the basis of their receiving either an excitatory or inhibitory vagal input from in-series tension receptors (Davison & Grundy, 1978). Thus the efferent response shows many characteristics of the afferent fibres with endings in the musculature of the stomach (Andrews, Grundy & Scratcherd, 1980*b*). Thus the efferent responses are slowly adapting to maintained inflation and, in the majority of cases, return rapidly to predistension frequencies of firing following deflation. Since the spontaneous discharge of the efferent fibres alters after vagotomy, decreasing in efferent units excited by gastric distension, and increases in units inhibited by distension, then the level of spontaneous discharge is also partially determined by the vagal afferent input.

Some of the efferent responses however, cannot be explained solely on receiving a vagal afferent input. As described above, many units receive a non-vagal and probably splanchnic input. In addition, the units which continue to discharge for long periods after the stomach is deflated must have some type of reverberating circuit which allows the discharge to continue after the afferent input has been reduced.

In respect of the afferent inputs from the duodenum and colon, a dramatic finding from these experiments was the extent of the non-vagal input onto the dorsal motor vagal nucleus. Over 80 % of all efferent units tested received a non-vagal input from the duodenum and colon, again indicating a sympatho–vagal reflex between the intestine and stomach. However, three efferent units showed evidence of some vagal

input from the colon, their response to colonic distension was partially reduced by bilateral vagotomy. Thus, although the major afferent input to these efferent units is via a spinal pathway, there is some evidence that a small proportion of afferent impulses travel via the vagus nerve.

Another surprising finding is the extent of the input onto individual vagal efferent fibres. In the present study, the majority of vagal efferent fibres received an input from the stomach, duodenum and colon. At present, no functional significance to these inputs can be made since a vital piece of missing information is the destination of the efferent fibres. That they innervate the gastrointestinal tract can be intimated from indirect criteria (Davison & Grundy, 1978). It is most likely that the fibres investigated were passing to the abdomen as those with respiratory and cardiovascular rhythms were rejected, and from histological studies the majority of non-myelinated efferent fibres are destined to innervate abdominal viscera (Agostini *et al.* 1957). However, within the gastrointestinal tract many possible functions regarding both secretion and motility exist. the fibres which continue to discharge following deflation of the stomach are most likely to have a secretory role since secretion will continue for long periods following afferent stimulation (Pavlov, 1902). The remaining fibres may serve to control motility. Because of the reciprocal nature of the efferent responses to gastric inflation, some being excited while others are inhibited, this could support the hypothesis of Miolan & Roman (1974) for reciprocal antagonism of vagal excitatory and inhibitory pathways.

One of the major effects of intestinal distension is inhibition of gastric motility and tone (Jansson, 1969). This has been described as being a pure sympathetic reflex with the efferent sympathetic outflow inhibiting at three levels, presynaptically onto preganglionic vagal cholinergic fibres (Hirst & McKirdy, 1974), onto myenteric cholinergic neurones (Jansson & Martinson, 1966) and directly onto the smooth muscle (for references see Bulbring, 1979). The present study suggests a fourth site for sympathetic inhibition of gastrointestinal motility at the level of the cell body for the vagal motoneurone within the dorsal motor vagal nucleus. The efferent units excited by colonic and duodenal distension may represent the preganglionic neurones to the non-cholinergic, non-adrenergic nerves which have been described as being activated during nociceptive stimulation of the duodenum (Abrahamsson, Glise & Glise, 1979). Distension stimuli arising in the gut therefore feed back onto preganglionic vagal neurones, inhibiting them and perhaps causing a reduction in the transit time through the gut.

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