Br. J. Pharmac. (1982), **77**, 419–429

EFFECTS OF ENKEPHALINS AND MORPHINE ON SPONTANEOUS ELECTRICAL ACTIVITY AND ON JUNCTION POTENTIALS ELICITED BY PARASYMPATHETIC NERVE STIMULATION IN CAT AND RABBIT COLON

FRANCE BLANQUET, MICHEL BOUVIER* & JEAN GONELLA*

Laboratoire de Physiologie, Faculté de Médecine, Boulevard Jean Moulin, 13385 Marseille, Cedex 4 and Département de Neurophysiologie végétative*, Institut de Neurophysiologie et Psychophysiologie C.N.R.S., 31 Chemin Joseph Aiguier B.P. 71, 13277 Marseille Cedex 9, France

1 The effects of Leu-enkephalin, Met-enkephalin and morphine on excitatory junction potentials (e.j.ps) and inhibitory junction potentials (i.j.ps) elicited by stimulation of efferent parasympathetic nerves were studied in cats and rabbits, anaesthetized and *in vitro*.

2 Enkephalins (0.008 mg/kg *in vivo* and 10^{-6}M *in vitro*) enhanced e.j.p. amplitude in rabbit proximal colon and decreased it in rabbit distal colon and in cat colon. Enkephalins decreased i.j.p. amplitude in all the three models.

3 Morphine (0.2 mg/kg *in vivo* and 10^{-6}M *in vitro*) had the same effects as enkephalins on e.j.ps. In contrast, morphine decreased i.j.p. amplitude in rabbit proximal and distal colon and increased it in cat colon.

4 Enkephalins and morphine induced (especially in the cat) spike activity which was potentiated by atropine (0.1 mg/kg *in vivo* or 10^{-6}M *in vitro*).

5 All the effects of enkephalins and morphine were antagonized by naloxone (0.2 mg/kg *in vivo* or 10^{-6}M *in vitro*).

6 These results suggest that the facilitatory effects of enkephalins and morphine on e.j.ps of rabbit proximal colon are due to the absence of opiate receptors on the excitatory nerve pathway and to a removal of inhibition by blockade of the non-adrenergic, non-cholinergic inhibitory pathway. Enkephalinergic intramural neurones may modulate the activation of either excitatory or inhibitory pathways in intramural reflexes.

Introduction

The existence of enkephalin-containing neurones in the intramural nervous system of the gut has been demonstrated by numerous immunohistochemical studies on various species (Furness, Costa, Franco & Llewellyn-Smith, 1980; Schultzberg, Hokfelt, Nilsson, Terenius, Rehfeld, Brown, Elde, Goldstein & Said, 1980). Extra- and intracellular recordings from guinea-pig and cat enteric neurones show that the rate of neuronal discharge is decreased by enkephalins and morphine which act on specific receptors. The mechanisms of action of morphine and enkephalins are complex since both cell bodies and nerve processes may be involved (North & Tonini, 1977; North, Katayama & Williams, 1979; Williams & North, 1979; Wood, 1980; Morita & North, 1981).

The aim of the present work was to determine the function of intramural enkephalinergic neurones in intestinal motility and whether their role was identical in different segments of the gut. A study of the effects of enkephalins and morphine has been made on (a) the spontaneous electromyographic activity (EMG) and (b) the amplitude of excitatory or inhibitory junction potentials (e.j.ps or i.j.ps) elicited by electrical stimulation of preganglionic parasympathetic nerve fibres.

Three experimental models were used: the cat colon, the rabbit distal colon, both innervated by the pelvic nerves, and the rabbit proximal colon which receives its efferent nerve supply from the vagus nerves (Jule & Gonella, 1972; Gonella & Gardette, 1974; Jule, 1975; 1980).

Methods

Animals

Adult cats and rabbits were used for *in vivo* experiments; the animals were fasted 24 h before the beginning of the experiment.

0007-1188/82/110419-11 $01.00

In vivo *experiments*

The experimental method was essentially that described by Bouvier & Gonella (1981a,b). Animals were anaesthetized with halothane (halothane 2%, air 98%), the trachea cannulated and a polyethylene catheter inserted into the radial (cat) or an ear (rabbit) vein. Halothane was progressively reduced and stopped while the animals were perfused intravenously with a Na thiopentone (Nesdonal, SPECIA) solution given by a peristaltic pump (20 mg kg^{-1} h^{-1}). It was established in test experiments that such doses of anaesthetic allowed a convenient level of anaesthesia, since the palpebral reflex was absent. In addition, cardiac rhythm and blood pressure were systematically recorded throughout the experiments in order to detect any variation of the anaesthesia level. Gallamine was added to the anaesthetic solution and the animals were artificially ventilated. The muscle relaxant ensured that regular respiratory movements were obtained which decreased the incidence of artifacts due to breathing in both species and it also avoided contraction of the tail muscles of the cat. The colon was exposed by laparotomy on the midline in the rabbit and on the right part of the abdomen in the cat. Body temperature was maintained at 37°C with a thermostatically controlled heating device.

Stimulating electrodes included in a plexiglas gutter were connected to a stimulator. In the cat, the sacral ventral roots (mainly S2) containing the efferent fibres of the pelvic nerves which innervate the colon were dissected after laminectomy and divided and the peripheral end of the root stimulated. In the rabbit, both vagi, which innervate the proximal colon, were dissected in the neck, divided and the distal ends stimulated.

In both preparations, the EMG was recorded with extracellular suction electrodes. Electrical recording was monopolar (a.c. amplifiers, time constant 2.5 s). EMG was recorded with an ink pen writer, an upward deflection corresponded to a positive value of the focal electrode.

In vitro *experiments*

After a brief induction with halothane (halothane 2%, air 98%) cats were anaesthetized with Na thiopentone intravenously (thiopentone 10% in 0.9% w/v NaCl solution). The distal part of the colon (12 to 15 cm from the anus) was excised together with both pelvic nerves, opened longitudinally on the mesenteric border, and the mucosa was removed.

In the rabbit, laminectomy being difficult to perform, the EMG of the distal colon was recorded *in vitro* only. The dissection was similar to that described for the cat, but the colon was excised after the animals had been killed by a blow on the neck and bled.

For both preparations the colon was placed in an organ bath perfused with a saline solution of the following composition (mmol): NaCl 133, KCl 4.7, $CaCl_2$ 1.9, $MgCl_2$ 0.49, NaH_2PO4 1.0 and glucose 7.8. The solution was bubbled with 95% O_2 and 5% CO_2 gas mixture and maintained at 35°C.

The nerves were stimulated with a device, described in detail by Jule & Gonella (1972), which allowed stimulation of the nerves inside the organ bath without any diffusion of the stimulus to the colonic wall.

The EMG was recorded with extracellular pressure electrodes (Gonella, 1972). Electrical recording was monopolar.

Statistical evaluations

Statistical analysis was performed using Student's test for paired samples. When the value of P was greater than 0.05, the difference was considered as non-significant. The mean values of junction potential amplitudes are given with the standard error of the mean (s.e.mean).

Drugs

The following drugs were used: enkephalins (Serva), morphine hydrochloride (Coop. Pharmaceutique Française), atropine sulphate (Merck), hexamethonium chloride (Fluka), propranolol hydrochloride (Sigma); phentolamine hydrochloride and guanethidine sulphate were gifts from CIBA and naloxone hydrochloride from Endo.

Results

Analysis of results

Nerve stimulation frequency was 2/min. Values of e.j.p. and i.j.p. amplitudes were ranged by classes for which mean amplitude and standard error were measured. With extracellular recordings the amplitude of EMG components varied from one recording to another. This was mainly due to the nature of the contact between electrode and tissue and to the thickness of serosa. The amplitude of e.j.ps and i.j.ps depended also on the position of the nerves on stimulating electrodes and could thus be compared only at the same site of recording. Therefore, in the graphs, variations in amplitude of electrical responses to nerve stimulation are given in arbitrary units as a function of time (Figures 3 and 5). The facilitatory effect of the enkephalins and morphine on the e.j.ps of the rabbit proximal and distal colon was difficult to measure. The general consequence of an increase in the amplitude of e.j.ps is the initiation of a spike potential. The rate of rise of the response is

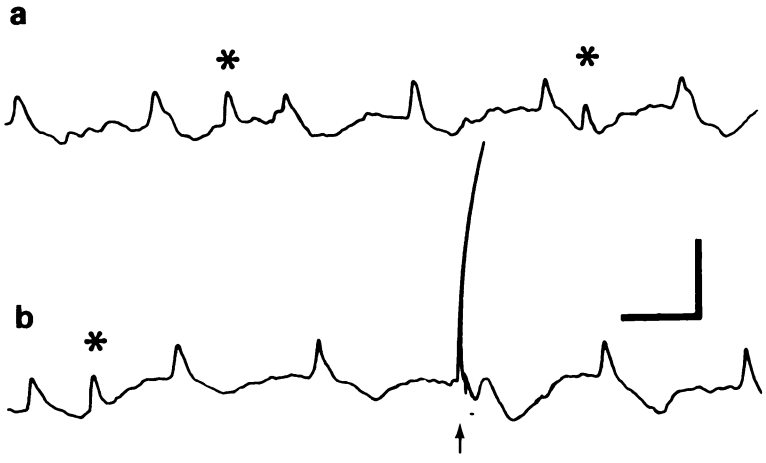

Figure 1 Action of Leu-enkephalin on e.j.ps of rabbit proximal colon: (a) control; (b) 1.2 min after injection of enkephalin (0.008 mg/kg); e.j.ps are indicated by asterisks, the other slow upward deflections are the slow waves characteristic of the rabbit proximal colon. On the control tracing, vagus nerve stimulation evokes only e.j.ps. After enkephalin, e.j.p. amplitudes are increased, the second e.j.p. initiates a spike potential (indicated by the arrow). Notice the absence of any inflexion point on the rising phase of the response, this does not allow the determination of the limit between the e.j.p. and the spike potential. Calibrations: 2 s; 0.5 mV. Stimuli: 2 shocks; 2 ms; 20 Hz; 10 V.

greatly increased and the limit between the top of the e.j.p. and the beginning of the spike potential becomes impossible to determine (Figure 1). The amplitude of the whole excitatory response was therefore measured. This allowed us to detect any increase in e.j.p. amplitude and to quantify it though e.j.ps and spike potentials depend on different physiological mechanisms. Our methods lead to an overestimation of the changes produced in the excitatory responses, i.e. in a highly excitable preparation, even a small increase in e.j.p. amplitude will initiate a spike potential involving a great variation in the amplitude of the response measured.

In cat and rabbit, *in vivo* experiments were performed generally on animals with an intact sympathetic innervation. Control experiments were done on cats with sectioned or even degenerated lumbar colonic nerves and in rabbits treated with guanethidine (2 mg kg^{-1} h^{-1}). In both cases no difference was observed as compared with normal animals indicating that the sympathetic nervous system was not involved in the phenomena described hereafter. In both species, the threshold dose of the enkephalins was 0.004 mg/kg *in vivo* and 10^{-7}M *in vitro* and for morphine: 0.2 mg/kg and 10^{-7}M *in vivo* and *in vitro* respectively. The enkephalins and morphine did not induce any change in the pattern of the slow waves exhibited by cat colon or rabbit proximal colon. The action of the enkephalins on the e.j.ps and on the i.j.ps was more marked than that of morphine whereas on the spontaneous activity the reverse was true.

Effects of enkephalins on EMG activity

Rabbit In the proximal colon an injection of Met- or Leu-enkephalin (0.004–0.2 mg/kg) induced transient spike activity or increased the frequency of an existing spike discharge in 32% of cases ($n = 47$; 10 animals). These effects were not modified by atropine (2 mg kg^{-1} h^{-1}) or by hexamethonium (1.5 mg/kg) but were absent in animals pretreated with naloxone (0.2 mg/kg; $n = 19$; 4 animals). On the distal colon, both Met- and Leu-enkephalins (10^{-6}M) produced effects similar to those observed on the proximal colon, but the incidence of these effects was more frequent (57% of cases; $n = 26$; 11 animals). The effects on the distal colon were also antagonized by naloxone (10^{-6}M).

Cat *In vivo*, in the absence of existing electrical activity, injection of enkephalins (0.008–0.05 mg/kg) initiated slow waves with superimposed spike potentials (Figure 2a,b). When slow waves were present, Met- and Leu-enkephalins induced trains of spikes superimposed on existing slow waves. Spike initiation occurred in 59% of cases ($n = 17$; 11 animals). Spike activity, lasting about 5 min, was observed in atropinized animals (0.1 mg/kg) in 83% of cases ($n = 18$; 15 animals), it was not abolished by hexamethonium (1.5 mg/kg), but was antagonized by naloxone (0.2 mg/kg) (Figure 2c and d). Identical results were obtained *in vitro*.

Both *in vivo* and *in vitro*, in the presence of atropine

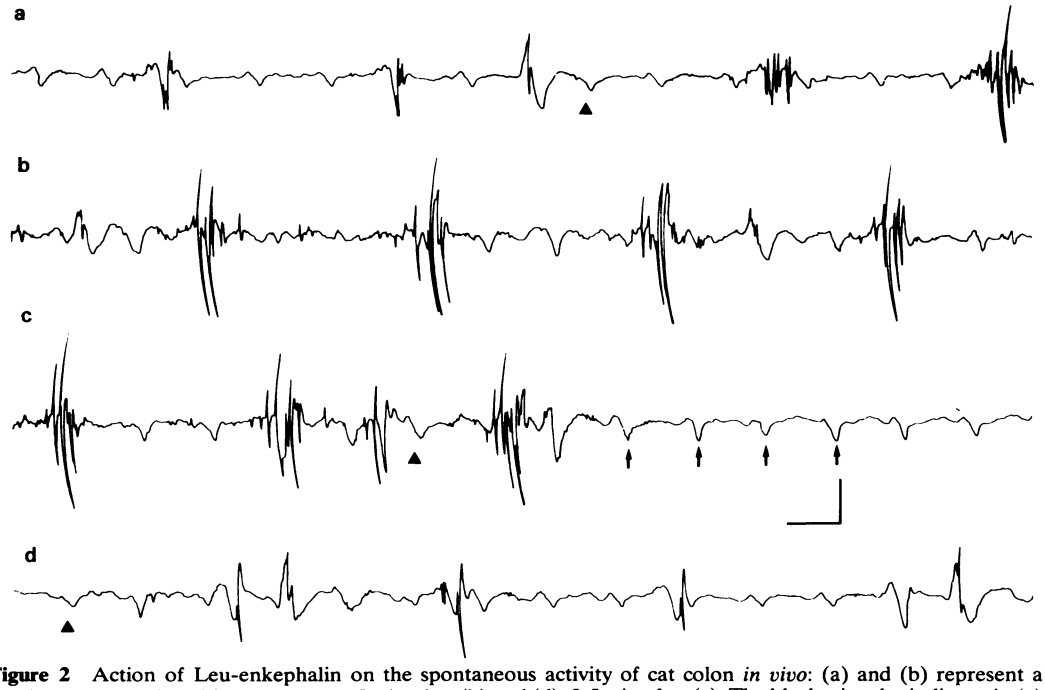

Figure 2 Action of Leu-enkephalin on the spontaneous activity of cat colon *in vivo*: (a) and (b) represent a continuous recording, (c) was recorded 5 min after (b) and (d), 2.5 min after (c). The black triangles indicate, in (a) an injection of enkephalin (0.008 mg/kg), in (c) an injection of naloxone (0.4 mg/kg), in (d) a second injection of enkephalin (0.008 mg/kg). Notice, after the first injection of enkephalin, the appearance of trains of spike potentials. The spikes are virtually abolished by naloxone. The second injection of enkephalin fails to elicit any response. The artifacts, similar to those indicated by arrows, are movements due to breathing. Calibrations: 5 s; 1 mV.

(37% of cases; $n = 19$; 16 animals), the spike activity was cyclical, i.e. periods of intense spiking were followed by periods of rest which lasted about 20 min.

Effects of enkephalins on junction potentials elicited by stimulation of preganglionic parasympathetic fibres

Action of enkephalins on e.j.ps On the proximal colon of the rabbit, enkephalins (0.004–0.2 mg/kg *in vivo*, or 10^{-6}M *in vitro*) induced an immediate but transient increase in the amplitude of the e.j.ps (mean increase: 133%) (Figure 3,4). *In vitro*, the amplitude increased throughout the application of the drug. These results are in striking contrast with those obtained on the distal colon of the rabbit or on the colon of the cat in which the enkephalins decreased the amplitude and often abolished the e.j.ps. The mean decrease was 42% and 89% for the rabbit and the cat respectively (Figures 3,4).

Action of naloxone on e.j.ps The amplitude of the e.j.ps was unaffected by naloxone in the colon of the cat but decreased significantly in the proximal and distal colon of the rabbit. This inhibitory effect, accompanied *in vivo* by a rise in blood pressure, was adrenergic in nature since it was suppressed by guanethidine (2 mg/kg). It can be supposed that naloxone induces a release of noradrenaline from postganglionic sympathetic nerve endings which in turn blocks synaptic transmission in the excitatory intramural pathway (Jule & Gonella, 1972; Jule, 1975). This sympathomimetic effect of naloxone was also present *in vitro*, it was abolished by guanethidine (10^{-5}M) and by propranolol (10^{-6}M) but was less affected by phentolamine (10^{-6}M), indicating that noradrenaline released under the action of naloxone acted merely on beta adrenoceptors. Guanethidine had not, by itself, any detectable effect on the amplitude of e.j.ps. Therefore, to avoid the sympathomimetic effect due to naloxone administration, all the experiments on the rabbit were performed in the presence of guanethidine (2 mg/kg *in vivo*, or 10^{-5}M *in vitro*). In these conditions, the effects of enkephalins (0.025 mg/kg *in vivo*, or 10^{-6}M *in vitro*) were abolished by naloxone (0.5 mg/kg *in vivo*, or 10^{-6}M *in vitro*) (Figure 3; Figure 4). In the cat the inhibitory effect of enkephalins was also decreased markedly by naloxone (0.2 mg/kg *in vivo*, or 10^{-5}M *in vitro*) (Figures 3, 4).

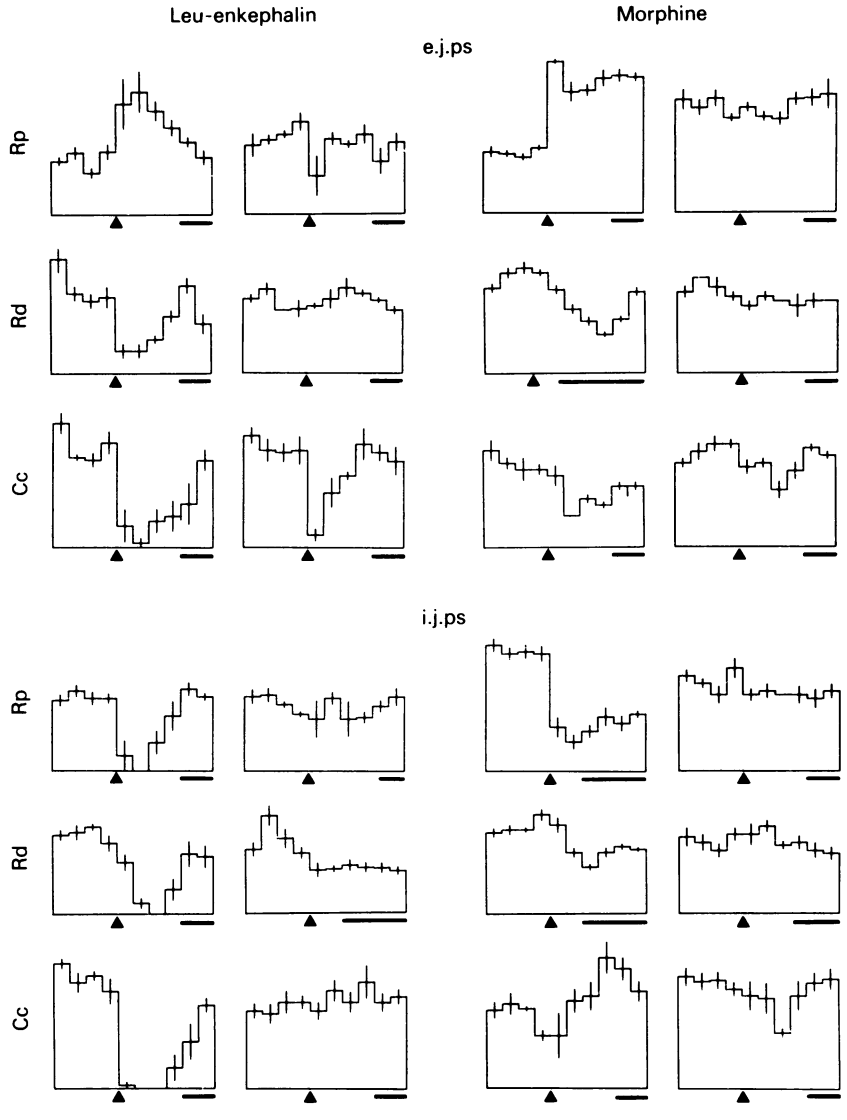

Figure 3 Effects of Leu-enkephalin and morphine (▲) on the amplitude of e.j.ps and i.j.ps evoked by stimulation of parasympathetic nerves on the rabbit proximal colon (Rp) and distal colon (Rd) and on cat colon (Cc). In each panel the left hand column represents the action of enkephalin or morphine, the right hand column that of the same drugs in the presence of naloxone (0.2 mg/kg *in vivo*: Rp and Cc; 10^{-6}M *in vitro*; Rd). The bar under each diagram represents 5 min. In each diagram, the amplitudes of several consecutive junction potentials in one experiment were pooled and presented as the mean with vertical lines indicating s.e.mean.

Action of enkephalins on i.j.ps In order to record i.j.ps without e.j.ps the experiments were performed under atropine (0.1 mg/kg for the cat; 2–3 mg/kg for the rabbit; 10^{-6}M *in vitro* for both species). In the three models studied here, i.j.ps were decreased markedly by enkephalins at doses similar to those previously found effective on e.j.ps (Figures 3, 4). In the rabbit, the mean decrease in i.j.p. amplitude was 81% and 77% for the proximal and distal colon respectively. In the cat the mean decrease was 65%. This inhibitory effect was markedly decreased by naloxone (0.2 mg/kg or 10^{-6}M) (Figures 3, 4). Under naloxone the decrease in amplitude was only of 32%.

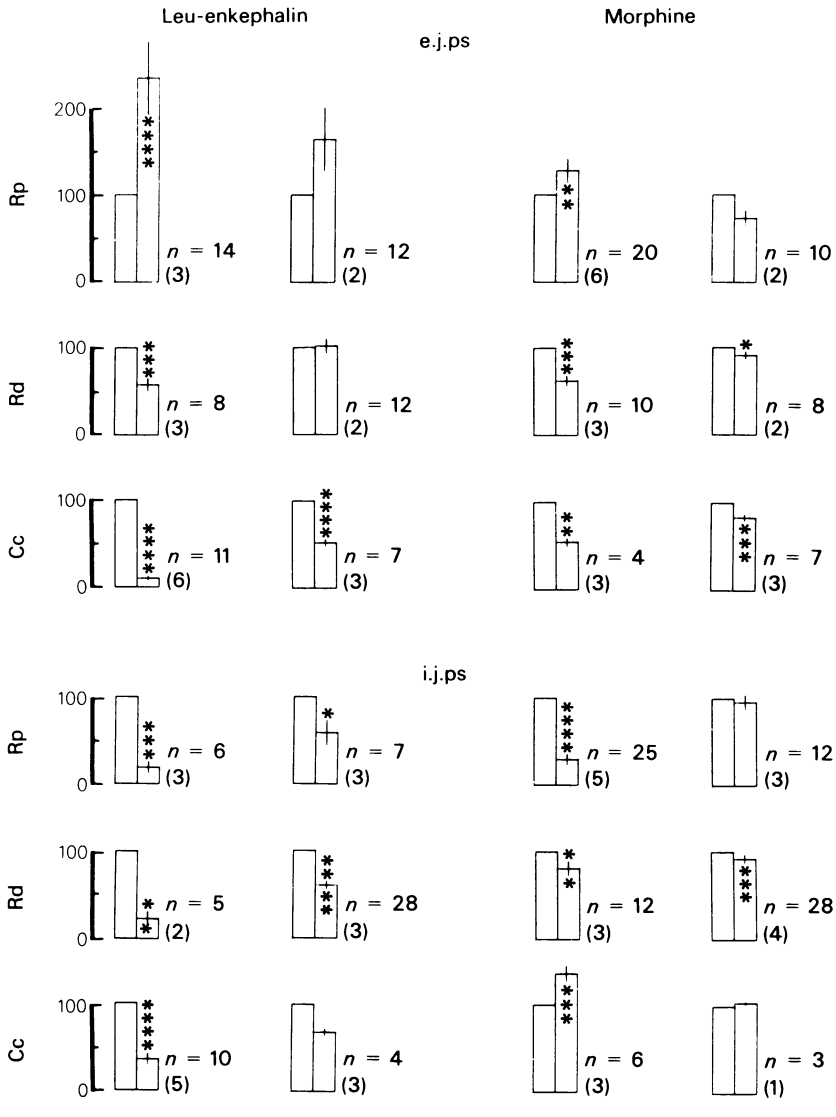

Figure 4 Modification of e.j.p. and i.j.p. amplitude by Leu-enkephalin and morphine, and by the same drugs plus naloxone. Rp: rabbit proximal colon; Rd: rabbit distal colon; Cc: cat colon. In each pair the mean control amplitude (left column) was taken as 100%; the right column represents the action of drugs. The vertical line on the top of each right column is the s.e.mean. Results significantly different from control are indicated by: $^{****}P<0.001$; $^{***}P<0.01$; $^{**}P<0.02$; $^{*}P<0.05$; columns without asterisks correspond to non-significant variations; *t* test for paired samples. *n* indicates the number of tests: the number of animals tested is given in parentheses.

Effects of morphine on EMG activity

The effects of morphine lasted longer than those of the enkephalins, since enkephalins undergo degradation more rapidly than morphine (Hughes, 1975).

In the rabbit On the proximal colon, morphine (0.2–1.5 mg/kg) induced either a spike potential activity or increased the frequency of existing spikes, in 36% of cases ($n = 55$). This excitatory effect lasted from 35 s to 20 min. In animals previously treated with naloxone (0.2 mg/kg) the excitatory effect of morphine was absent in 86% of cases ($n = 22$) or was markedly decreased (13% of cases) compared with control. The excitatory effect of morphine was unaffected by atropine or by hexamethonium

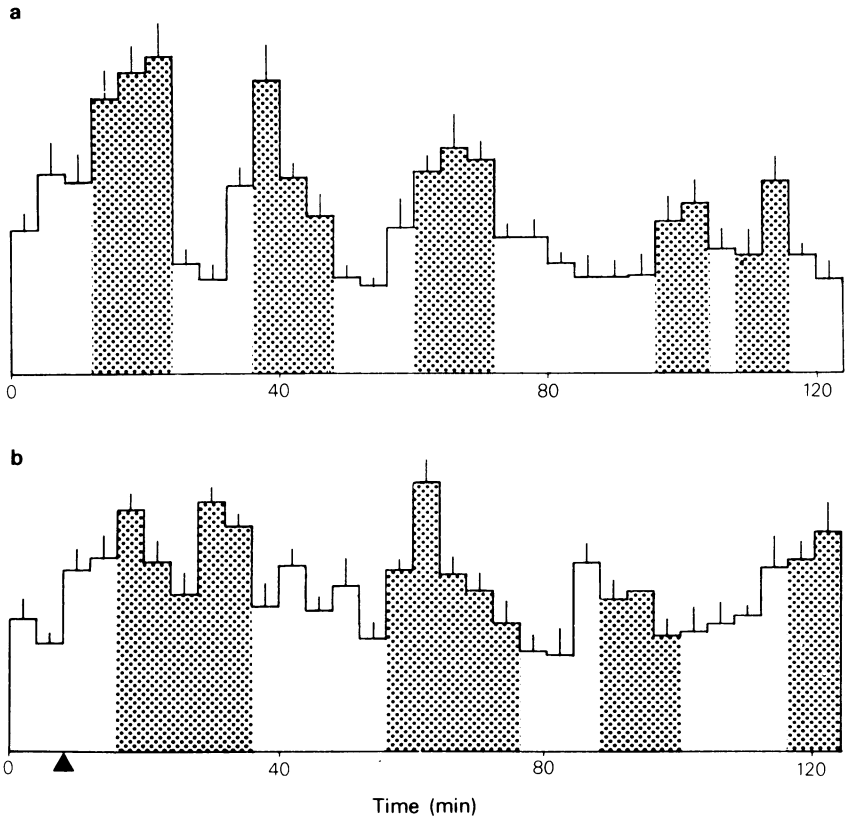

Figure 5 Fluctuations in i.j.p. amplitude, as a function of time, occurring in two different experiments on cat colon, in presence of atropine (0.1 mg/kg). The variations occurred spontaneously in (a) and under the action of morphine in (b). The amplitudes of i.j.ps were pooled and presented as the mean ± s.e.mean. The stippled zones indicate the occurrence of spike potentials. The black triangle in (b) indicates the time of morphine injection (0.2 mg/kg).

(2 mg kg^{-1} h^{-1} and 1.5 mg/kg respectively). On the distal colon, morphine (10^{-6}M) had an excitatory effect in 60% of cases ($n = 30$) which was suppressed by naloxone (10^{-6}M) in 78% of cases and markedly decreased in the others ($n = 44$).

In the cat Morphine (0.2–1 mg/kg *in vivo*, 10^{-6}M–10^{-5}M *in vitro*) had similar but longer lasting effects than those of the enkephalins, i.e. an increase in spiking activity in 74% of cases. In the presence of atropine (0.1 mg/kg *in vivo*, or 10^{-6}M *in vitro*) enhancement of spike activity occurred in all instances and became cyclic in 70% of cases, i.e. it consisted of trains of spikes lasting from 8 to 20 min interrupted by irregular periods of electrical silence lasting from 8 to 25 min. The spike discharge was significantly decreased by naloxone (0.2 mg/kg), but was unaffected by hexamethonium (1 mg/kg). In one experiment, with atropine, cyclic activity occurred spontaneously, i.e. in the absence of morphine (Figure 5a). In another experiment, in the absence of atropine, morphine induced cyclic activity.

Effects of morphine on junction potentials elicited by stimulation of preganglionic parasympathetic fibres

Effects on the e.j.ps In the three models studied the effects of morphine were very similar to those of the enkephalins, i.e. an increase in i.j.p. amplitude on the rabbit proximal colon and a decrease in e.j.p. amplitude on the rabbit distal colon and on the cat colon. The increase in amplitude of the excitatory responses was 28% for the rabbit proximal colon, whereas the decrease in amplitude was 38% and 45% for the distal colon of the rabbit and the colon of the cat respectively (Figures 3, 4). In the three models used in these experiments the action of morphine was blocked by naloxone (0.2 mg/kg *in vivo*, or 10^{-6}M *in vitro*) (Figures 3, 4).

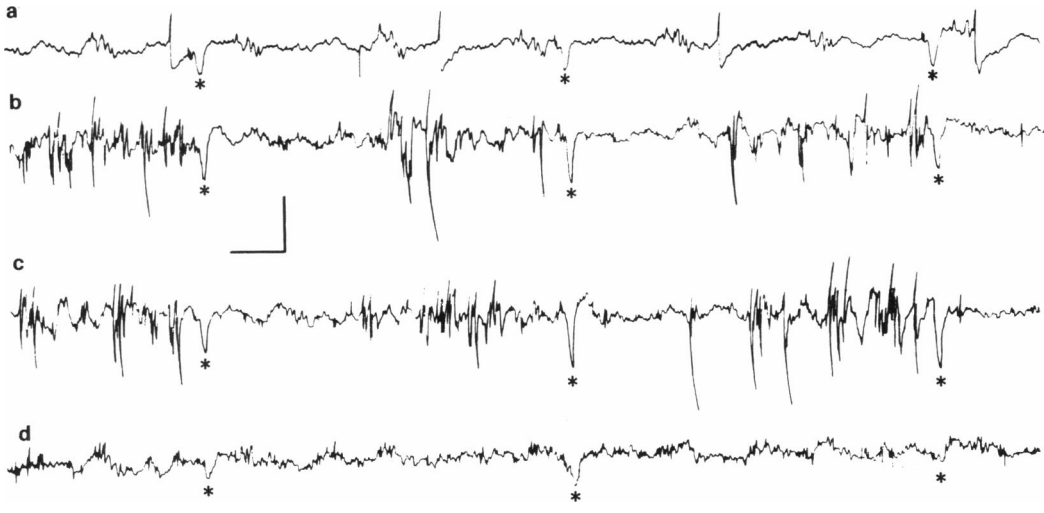

Figure 6 Increase in i.j.p. amplitude during spike activity observed in cat colon under morphine: i.v. injection of morphine (0.2 mg/kg) was given 11 min before the beginning of (a) (animal treated with atropine, 0.1 mg/kg); (a), (b), (c) and (d) tracings are not continuous records. i.j.ps are indicated by asterisks. Notice the increase in i.j.p. amplitude in (b) and (c) during the occurrence of spike potentials. Some of the spike potentials which are recorded in close vicinity of the electrode have positive initial phase as in (a), others which are initiated farther from the electrode, probably in the circular layer, are polyphasic, with a negative main phase, as in (b) and (c). Calibrations: 5 s; 1 mV. (Stimuli: 4 pulses; 2 ms; 20 Hz; 25 V).

Effects on i.j.ps In the rabbit proximal and distal colon i.j.p. amplitude was decreased by morphine, whereas on the cat colon it was slightly increased (Figures 3, 4). In addition, in the cat, recordings made over a prolonged period (about 2 h) indicated that i.j.p. amplitude fluctuated periodically (Figure 5b). The maximum i.j.p. amplitude coincided approximately with the maximum period of spike discharges (Figure 5b, 6). The action of morphine on i.j.ps was decreased by naloxone (Figures 3, 4).

Action of enkephalins and morphine on the post-inhibitory activation

In the rabbit proximal colon, under atropine, i.j.ps were generally followed by a depolarization with one

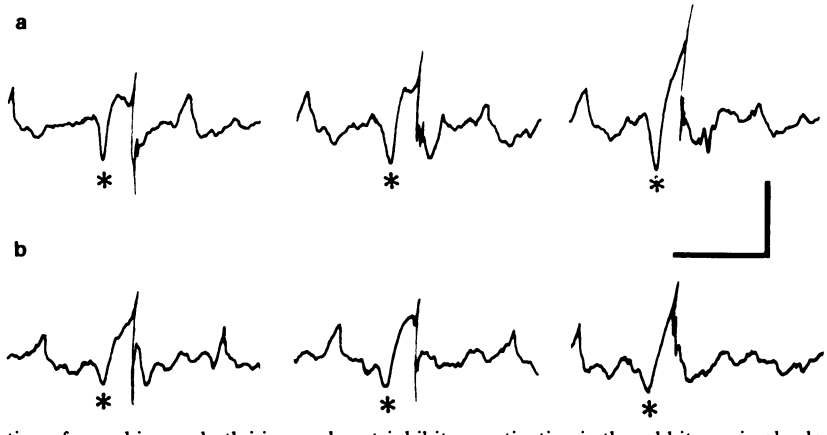

Figure 7 Action of morphine on both i.j.ps and post-inhibitory activation in the rabbit proximal colon elicited by vagal stimulation (5 pulses; 2 ms; 20 Hz; 30 V). (a) Three consecutive control responses; (b) 5, 6 and 9 min respectively after i.v. injection of morphine 1 mg/kg. Notice that, after morphine, i.j.p. amplitude is markedly reduced while the amplitude of the excitatory response remains virtually unchanged. Calibrations: 5 s; 1 mV.

or several superimposed spike potentials. This phenomenon has been observed neither on the rabbit distal colon nor on the cat colon. This excitation which follows the inhibitory response was much less influenced by enkephalins and morphine than the i.j.ps (Figure 7).

Discussion

Morphine reduces the release of acetylcholine from intramural neurones and decreases the activity of enteric neurones (Paton, 1957; Paton & Zar, 1968). This reduction in intramural neurone discharge has been confirmed by intracellular recordings in myenteric neurones of the intestine of both guinea-pig (North & Tonini, 1977; Williams & North, 1979; Morita & North, 1981) and cat (Wood, 1980). Morphine has also an indirect effect on the motility of small intestine by acting on the central nervous system (Stewart, Weisbrodt & Burks, 1976; Schulz, Wuster & Herz, 1979; Pascaud, Genton, Remond & Vincent, 1980; Weisbrodt, Thor, Anderson & Copeland, 1981).

The present results are due to a peripheral action, since they were observed on animals with sectioned extrinsic nerves as well as *in vitro*. In addition, they indicate that both the enkephalins and morphine act on opiate receptors since the responses are reversed by the specific opiate antagonist, naloxone.

The effects observed are probably due to an action of opiates on the intramural nerves since morphine has no direct action on smooth muscle cells as evidenced for the guinea-pig ileum by Ito & Tajima (1980). Enkephalins also act by preventing the release of acetylcholine from enteric neurones (Furness *et al.*, 1980; Morita & North, 1981).

Action of opiates on junction potentials

In both cat and rabbit, the efferent parasympathetic outflow to the colon is composed of cholinergic axons with two different functions. Some of these axons are connected with intramural cholinergic neurones which excite the smooth muscle cells, other are connected with intramural non-adrenergic non-cholinergic neurones (NANC) which inhibit the smooth muscle cells (the so-called purinergic neurones described by Burnstock, Campbell, Bennett & Holman, 1964). When the distal cut end of the pelvic or vagus nerve is stimulated, the excitatory pathway is generally activated and e.j.ps are recorded from smooth muscle cells; the e.j.ps may initiate spike potentials. When the excitatory pathway is blocked by atropine the response of smooth muscle cells consist of an i.j.p. (Jule & Gonella, 1972; Gonella & Gardette, 1974).

Action of opiates on i.j.ps in the rabbit

On the proximal and distal colon, both the enkephalins and morphine have a powerful inhibitory effect on the inhibitory pathway composed of preganglionic parasympathetic axons connected with NANC intramural inhibitory neurones. This result is in keeping with that of Shimo & Ishii (1978) who showed that morphine, following stimulation of parasympathetic preganglionic axons, decreased the relaxation of the guinea-pig taenia coli produced by excitation of NANC neurones. Opiates do not apparently act on the axons of NANC neurones since Ito & Tajima (1980) have shown that morphine does not abolish i.j.ps evoked by intramural nerve stimulation in the guinea-pig ileum. Opiates might act either on preganglionic nerve endings or on NANC cell bodies. Both the enkephalins and morphine block transmission in the enteric nervous system of guinea-pig small intestine either by hyperpolarizing the soma of enteric neurones (Wood, 1980) or by blocking conduction in nerve cell processes (Morita & North, 1981).

Action of opiates on e.j.ps in the cat colon and rabbit distal colon

In both models, opiates blocked the cholinergic excitatory pathway. Our results agree with those of Ito & Tajima (1980) who showed that in the guinea-pig ileum morphine prevented acetylcholine release from intramural cholinergic neurones and thus blocked excitation of smooth muscle cells.

Action of opiates on e.j.ps in the proximal colon of the rabbit

The facilitatory action of opiates on the e.j.ps of the proximal colon of the rabbit can be explained in two ways. One hypothesis is that opiates have a direct facilitatory effect on cholinergic neurones for instance by increasing the release of acetylcholine. A second, and more probable explanation, could be that the excitatory neurones of this part of the rabbit colon do not possess opiate receptors which would be present only on the inhibitory pathway. In normal conditions the result of vagal stimulation is the summation of the excitation of two types of preganglionic axons, i.e. excitatory and inhibitory ones. When the inhibitory pathway is blocked by opiates there is a removal of inhibition which in turn results in an increase of e.j.p. amplitude. Our results indicate that opiates act on the transmission of excitation either on the excitatory, or on the inhibitory pathway, or on both. From a functional point of view, this may be surprising but it may be that, in physiological conditions, only one of these pathways would be affected, whereas exogenous opiates would block both nerve

pathways at the same time.

On the basis of the fluctuation in the amplitude of e.j.ps and i.j.ps observed in the colon and the lower oesophagus of the cat, the existence of cross-connections between the intramural excitatory and inhibitory nerve pathways has been proposed (Gonella & Gardette, 1974; Gonella, Niel & Roman, 1977). Enkephalins may act as neurotransmitters in the rabbit small intestine (Oka, 1980); therefore the enkephalinergic intramural neurones might control impulse traffic passing through either one or other pathway.

Action of opiates on EMG activity

Spiking activity induced by the enkephalins and morphine is similar to the motor activity described in the rat colon by Gillan & Pollock (1980). This effect, antagonized by naloxone, is specific for opiate receptors. A direct effect on smooth muscle is unlikely, morphine does not modify membrane potential in the guinea-pig ileum (Ito & Tajima, 1980). A comparable result has been obtained with morphine and the enkephalins on cat colon, using the sucrose gap method (Bouvier, personal communication).

Removal of inhibition by blockade of NANC neurones can be also rejected, since in the cat i.j.p. amplitude is highest at a time when the spike frequency is highest. Thus it seems that both the action of opiates on i.j.ps and the initiation of intense spike activity are independent phenomena. Therefore, the spiking activity produced by opiates could be due to excitation of intramural non-cholinergic excitatory neurones (see Ambache & Freeman, 1968; Furness & Costa, 1973; Fasth, Hulten & Nordgren, 1980).

Nature of the post-stimulus depolarization in atropinized rabbits

The excitation which follows the i.j.ps in atropinized rabbit proximal colon, has been considered in the past as a post-inhibitory rebound. The mechanisms of this phenomenon remains controversial; however, it appears that the hyperpolarization (i.e. the i.j.p.) and the following depolarization are presumably two independent phenomena due to the release of two different neurotransmitters (see Bywater, Holman & Taylor, 1981). Our results are in keeping with such an explanation, since opiates can decrease considerably i.j.p. amplitude without affecting the atropine-resistant post-stimulus depolarization. The nature of the atropine-resistant neurotransmitter remains to be determined.

We wish to express our thanks to Dr T.C. Muir for his valuable critical reading of the manuscript and his suggestions. We appreciated the helpful assistance of Mrs F. Farnarier in the preparation of this manuscript. We are also indebted to Dr. M. Dufossé for his help in computorized treatment of the data. We thank Mrs P. Rouvière for her skilled technical assistance. Reprint requests to M.B. please.

References

AMBACHE, N. & FREEMAN, M.A. (1968). Atropine-resistant longitudinal muscle spasms due to excitation of non-cholinergic neurones in Auerbach's plexus. *J. Physiol.*, **199,** 705–727.

BOUVIER, M. & GONELLA, J. (1981a). Electrical activity from smooth muscle of the anal sphincteric area of the cat. *J. Physiol.*, **310,** 445–456.

BOUVIER, M. & GONELLA, J. (1981b). Nervous control of the internal anal sphincter of the cat. *J. Physiol.*, **310,** 457–469.

BURNSTOCK, G., CAMPBELL, G., BENNETT, M.R. & HOLMAN, M.E. (1964). Innervation of the guinea-pig taenia coli: are there intrinsic inhibitory nerves which are distinct from sympathetic nerves? *Int. J. Neuropharmac.*, **3,** 163–166.

BYWATER, R.A.R., HOLMAN, M.E. & TAYLOR, G.S. (1981). Atropine-resistant depolarization in the guinea-pig small intestine. *J. Physiol.*, **316,** 369–378.

COSTA, M. & FURNESS, J.B. (1976). The peristaltic reflex: An analysis of the nerve pathways and their pharmacology. *Naunyn-Schmiedebergs Arch. Pharmac.*, **294,** 47–60.

FASTH, S., HULTEN, L. & NORDGREN, S. (1980). Evidence for a dual pelvic nerve influence on large bowel motility in the cat. *J. Physiol.*, **298,** 159–169.

FURNESS, J.B. & COSTA, M. (1973). The nervous release and the action of substances which affect intestinal muscle through neither adrenoreceptors nor cholinoreceptors. *Phil. Trans. R. Soc., B,* **265,** 123–133.

FURNESS, J.B., COSTA, M., FRANCO, R. & LLEWELLYN-SMITH, I.J. (1980). Neuronal peptides in the intestine: distribution and possible functions. In *Neural Peptides and Neuronal Communication.* ed Costa, & Trabucchi, M. New York: Raven Press.

GILLAN, M.G.C. & POLLOCK, D. (1980). Acute effects of morphine and opioid peptides on the motility and responses of rat colon to electrical stimulation. *Br. J. Pharmac.*, **68,** 381–392.

GONELLA, J. (1972). Modifications of electrical activity of the rabbit duodenum longitudinal muscle after contractions of the circular muscle. *Am J. dig. Dis.*, **17,** 327–332.

GONELLA, J. & GARDETTE, B. (1974). Etude electromyographique in vivo de la commande nerveuse extrinsèque parasympathique du côlon. *J. Physiol., Paris,* **68,** 395–413.

GONELLA, J., NIEL, J.P. & ROMAN, C. (1977). Vagal control of lower oesophageal sphincter motility in the cat. *J. Physiol.*, **273,** 647–664.

HUGHES, J. (1975). Isolation of an endogenous compound

from the brain with pharmacological properties similar to morphine. *Brain Res.*, **88,** 295–308.

ITO, Y. & TAJIMA, K. (1980). Action of morphine on the neuro-effector transmission in the guinea-pig ileum and in the mouse vas deferens. *J. Physiol.*, **307,** 367–383.

JULE, Y. (1975). Modifications de l'activité électrique du côlon proximal de Lapin in vivo, par stimulation des nerfs vagues et splanchniques. *J. Physiol., Paris*, **70,** 5–26.

JULE, Y. (1980). Nerve-mediated descending inhibition in the proximal colon of the rabbit. *J. Physiol.*, **309,** 487–498.

JULE, Y. & GONELLA, J. (1972). Modifications de l'activité électrique du côlon terminal de Lapin par stimulation des fibres nerveuses pelviennes et sympathiques. *J. Physiol., Paris*, **64,** 599–621.

MORITA, K. & NORTH, R.A. (1981). Opiates and enkephalin reduce the excitability of neuronal processes. *Neuroscience*, **6,** 1943–1951.

NORTH, R.A., KATAYAMA, Y. & WILLIAMS, J.T. (1979). On the mechanism and site of action of enkephalin on single myenteric neurons. *Brain Res.*, **165,** 67–77.

NORTH, R.A. & TONINI, M. (1977). The mechanism of action of narcotic analgesics in the guinea-pig ileum. *Br. J. Pharmac.*, **61,** 541–549.

OKA, T. (1980). Enkephalin receptor in the rabbit ileum. *Br. J. Pharmac.*, **68,** 193–195.

PASCAUD, X.B., GENTON, M.G., REMOND, G. & VINCENT, D. (1980). Antral to colonic motility responses to intracerebroventricular administration of D-Ala-2-enkephalinamide, beta-endorphin, methionine-enkephalin and fentanyl in anesthetized rats. In *Gastrointestinal Motility*. ed Christensen J. New York: Raven Press.

PATON, W.D.M. (1957). The action of morphine and related substances on contractions and on acetylcholine output of coaxially stimulated guinea-pig. *Br. J. Pharmac.*, **11,** 119–127.

PATON, W.D.M. & ZAR, A.M. (1968). The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle strips. *J. Physiol.*, **194,** 13–33.

SCHULZ, R., WUSTER, M. & HERZ, A. (1979). Centrally and peripherally mediated inhibition of intestinal motility by opioids. *Arch. Pharmac.*, **308,** 255–260.

SCHULTZBERG, M., HOKFELT, T., NILSSON, G., TERENIUS, L., REHFELD, J.F., BROWN, M., ELDE, R., GOLDSTEIN, M. & SAID, S. (1980). Distribution of peptide- and catecholamine-containing neurons in the gastro-intestinal tract of rat and guinea-pig: Immunohistochemical studies with antisera to substance P, vasoactive intestinal polypeptide, enkephalins, somatostatin, gastrin/cholecystokinin, neurotensin and dopamine betahydroxylase. *Neuroscience*, **5,** 689–744.

SHIMO, Y. & ISHII, T. (1978). Effects of morphine on non-adrenergic inhibitory responses of the guinea-pig taenia coli. *J. Pharm. Pharmac.*, **30,** 596–597.

STEWART, J.J., WEISBRODT, N.W. & BURKS, T.F. (1976). Intestinal reverse peristalsis associated with morphine-induced vomiting. In *Opiates and Endogenous Opioid Peptides*. pp. 439–442. Amsterdam: Elsevier.

STEWART, J.J., WEISBRODT, N.W. & BURKS, T.F. (1977). Centrally mediated intestinal stimulation by morphine. *J. Pharmac. exp. Ther.*, **202,** 174–181.

WEISBRODT, N.W., THOR, P.J., ANDERSON, J. & COPELAND, E.M. (1981). Morphine's effect on intestinal motility is mediated via the central nervous system (CNS). *Zeitschrift für Gastroenterologie*, **19,** 412.

WILLIAMS, J.T. & NORTH, R.A. (1979). Effects of endorphins on single myenteric neurons. *Brain Res.*, **165,** 57–65.

WOOD, J.D. (1980). Intracellular study of effects of morphine on electrical activity of myenteric neurons in cat small intestine. *Gastroenterology*, **79,** 1222–1230.

(Received March 19, 1982. Revised June 16, 1982.)