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ACUTE EFFECTS OF MORPHINE AND OPIOID PEPTIDES ON THE MOTILITY AND RESPONSES OF RAT COLON TO ELECTRICAL STIMULATION

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1 Morphine and leucine- and methionine-enkephalins inhibited the contractile response of the pithed rat colon to electrical stimulation of the spinal motor outflows and inhibited motor responses of the isolated colon to field stimulation.

2 Morphine and the opioid peptides also had an excitatory action in the colon. In the pithed rat, opiates caused regular fluctuations in intracolonic pressure and in the isolated colon, caused regular waves of contraction. This excitatory response was produced by low concentrations of the enkephalins (2×10^{-8} M, 2×10^{-9} M), was stereospecific and was antagonized by naloxone.

3 Opiate-induced contractions in the isolated colon were inhibited by catecholamines, adenine nucleotides and by phosphodiesterase inhibitors. These contractions were unaffected by ergotamine and tolazoline, or by propranolol.

4 The excitatory action of opiates in the isolated colon was not antagonized and usually was potentiated by atropine, (+)-tubocurarine and hexamethonium. In the absence of opiates, these drugs also produced similar waves of contraction, which were unaffected by naloxone.

5 Opiate-induced contractions occurred in colon rendered unresponsive to 5-hydroxytryptamine (5-HT) and these contractions were potentiated by the 5-HT antagonist, lysergic acid diethylamide, which, when administered alone, caused similar contractions. The 5-HT antagonist, cyproheptadine, inhibited opiate-induced contractions but was non-specific, since it also inhibited responses of the colon to carbachol and KCl.

6 Opiate-induced contractions were unaffected by procaine and were potentiated by tetrodotoxin. Both of these drugs, when administered alone, produced waves of contractions, which were similar to those produced by opiates but were unaffected by naloxone.

7 Contractions produced in the isolated colon either by opiates, atropine or (+)-tubocurarine, or any combination of these drugs, were inhibited by field stimulation applied at the peak of a wave of contraction. This inhibitory response to field stimulation occurred at low frequencies of stimulation (< 10 Hz), and persisted in colon from rats pretreated with reserpine to deplete, or 6-hydroxydopamine to destroy, adrenergic nerve endings. It was unaffected by guanethidine but abolished by tetrodotoxin.

8 The implications of these results are considered and it is concluded that the excitatory action of opiates in the rat colon is probably not mediated by the release of acetylcholine or 5-HT but instead, may be due either to a direct action on smooth muscle or to a presynaptic inhibitory action at a ganglionic site in a non-adrenergic inhibitory mechanism, which normally suppresses myogenic activity.

Introduction

The effects of morphine on the motility of the gastrointestinal tract have been widely studied and vary between species and according to the dose, the route of administration and the method of assessment used (Vaughan Williams, 1954). Generally, morphine increases the tone and intraluminal pressure but decreases the propulsive activity of the gastrointestinal

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tract (Jaffe & Martin, 1975). The mechanisms underlying these effects are still poorly understood but may involve central (Weinstock, 1971) or, perhaps more likely, peripheral actions of morphine on enteric nerves (Burks & Long, 1967).

There is evidence that morphine interferes with both the storage and release of acetylcholine (ACh) from parasympathetic nerves in the intestine. For example, in the guinea-pig ileum, morphine inhibits the release of ACh at the neuroeffector junction (Paton, 1957). In contrast, morphine may increase the contractility of dog intestine by releasing 5-hydroxytryptamine (5-HT) and neuronal ACh, the latter being displaced indirectly by the former (Burks, 1973).

The effects of morphine in the gastrointestinal tract, on the metabolism of transmitters other than ACh, are less clear. At least two additional transmitters exist in the bowel wall. These are noradrenaline, the transmitter of the postganglionic adrenergic inhibitory nerves, which end mainly around ganglion cells (Norberg, 1964; Furness & Costa, 1974), and the less well documented transmitter of the intramural non-adrenergic, non-cholinergic inhibitory nerves, which may be 'purinergic' (Burnstock, 1975).

The significance of the effects of morphine on neurohumoral transmission in the intestine has now increased with the discovery that the opioid peptides, leucine- and methionine-enkephalin occur in the intestine (Elde, Hokfelt, Johansson & Terenius, 1976), and more significantly, are localized in the myenteric plexus in a way which suggests that they might have a neurotransmitter or neuromodulator role in the enteric nervous system (Kosterlitz & Hughes, 1975; Smith, Hughes, Kosterlitz & Sosa, 1976; Hughes, Kosterlitz & Smith, 1977).

The purpose of this study was to examine the effects of morphine and these opioid peptides on the motility and responses of the rat colon to electrical stimulation, and to determine whether these effects could be explained by an action on cholinergic or any other neuronal mechanisms in the intestine.

The pithed rat was used to investigate the effects of morphine and enkephalins on the motility of the colon *in situ*, because these effects could be investigated in this preparation without any superimposed effects of an anaesthetic complicating interpretation of the results. This preparation also permitted investigation of the effects of the opiates on responses of the colon to electrical stimulation of the spinal autonomic nerves supplying it.

In vitro studies, involving field stimulation of isolated segments of colon, permitted a more detailed analysis to be made of the effects of opiates on the motility of the rat colon.

A preliminary account of some of the work described in this paper has been presented to the British Pharmacological Society (Gillan & Pollock, 1976).

Methods

Experiments in the pithed rat

Recording intraluminal pressure changes in the colon of the pithed rat Male Wistar rats (230 to 270 g) were anaesthetized with Trilene, respired artificially at 90/min with a Palmer respiration pump, and pithed with a teflon-covered electrode, inserted through the orbit into the spinal canal by the method of Gillespie, MacLaren & Pollock (1970). The temperature of each rat was monitored with a rectal thermometer and maintained at 37°C by a tungsten lamp.

In each rat, one carotid artery and one femoral vein were cannulated. Arterial blood pressure was recorded by an Elcomatic pressure transducer (EM70) connected to the cannula in the carotid artery, and drugs were administered intravenously into the femoral vein. Before recording intraluminal pressure, the colon was emptied by gentle squeezing and washed out with saline (0.9% w/v NaCl solution). To record intraluminal pressure, a saline-filled polythene balloon was inserted into the colon to a depth of 3 to 4 cm. Intracolonic pressure changes were recorded with an Elcomatic (EM70) pressure transducer and displayed on a Grass polygraph together with the record of arterial blood pressure.

Stimulation of the spinal motor outflows to the colon in the pithed rat The spinal autonomic motor outflows to the colon were stimulated by the teflon-shielded electrode, lying within the spinal canal (Gillespie *et al.*, 1970). These outflows were stimulated electrically with supramaximal voltage pulses of 1 ms duration and variable frequencies, supplied by a Palmer stimulator. Skeletal muscle twitching caused by stimulation of the motor nerve fibres in the ventral roots was prevented by gallamine (5 mg/kg, i.v.). The position of the electrode within the spinal canal could be varied and was determined radiographically.

In vitro *experiments*

Recording mechanical activity of the isolated colon Male Wistar rats (230 to 270 g) were stunned and killed by bleeding. From each rat, one 3 to 4 cm length of terminal colon was excised and suspended in an organ bath containing 100 ml of Krebs bicarbonate solution (mM:NaCl 118.1, KCl 4.7, $MgSO_4$ 1.0, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0 and glucose 11.1), which was maintained at 37°C and gassed continuously with 95% O_2 and 5% CO_2.

An initial resting tension of 2 g was applied to each piece of colon and changes in the tension on the muscle were recorded isometrically by a Grass FTO3 force-displacement transducer and displayed on a Grass polygraph.

Field stimulation of the isolated colon The responses of segments of the rat colon to field stimulation of the intramural nerves were investigated. The colon was suspended in an organ bath containing Krebs solution at 37°C and stimulated electrically through platinum ring electrodes (Burn & Rand, 1960), with supramaximal voltage pulses of 0.2 ms duration and variable frequency, supplied either by a Palmer or a Grass S88 stimulator.

Simultaneous recording of electrical and mechanical activity in the colon Electrical activity within the wall of the colon was recorded by suction electrodes placed on the serosal surface of an intact segment of the colon, mounted horizontally in a shallow bath containing oxygenated Krebs bicarbonate solution at 37°C. One end of the colon was anchored to the base of the bath and the other was attached, via a pulley, to a Grass FTO3 force-displacement transducer. Electrical signals from the suction electrode were passed through an SRI AC preamplifier and displayed together with a record of the mechanical activity of the colon on a Grass polygraph.

Fluorescence microscopy

Pieces of colon from rats pretreated with reserpine to deplete, or 6-hydroxydopamine (6-OHDA) to destroy, adrenergic nerve endings, were examined with the Falck fluorescence technique (Falck, 1962) to ascertain whether these drug treatments had been effective. Tissues were prepared by the modified Falck technique described by Gillespie & Kirpekar (1966) and were examined with a Leitz Ortholux fluorescence microscope, using an HbO 200 mercury vapour lamp as light source, a 3 mm BG 12 filter to isolate the appropriate excitatory wavelength and a Leitz K 53 b barrier filter. Photomicrographs were taken on Ilford FP 4 film.

Comparison of the fluorescence in tissues from control, and from reserpine or 6-OHDA pretreated rats, confirmed that these treatments were effective in eliminating catecholamines from the colon.

Drugs and drug treatments

The drugs and treatments used in this investigation were acetylcholine chloride (ACh, Koch-Light), adenosine (Sigma), adenosine diphosphate (ADP, Sigma), adenosine monophosphate (AMP, Sigma), adenosine triphosphate (ATP, Sigma, adrenaline bitartrate (Sigma), ascorbic acid (Sigma), atropine sulphate (BDH), caffeine sodium salicylate (Allen & Hanburys), carbamyl choline chloride (carbachol, Sigma), carboxypeptidase A (Sigma), cyproheptadine (Merck, Sharp & Dohme), dextrorphan tartrate (Roche), dibutyryl cyclic adenosine monophosphate (cyclic AMP, Sigma), ergotamine tartrate (Sandoz), gallamine triethiodide (May & Baker), guanethidine monosulphate (Ciba), hexamethonium bromide (C_6, May & Baker), 6-hydroxydopamine hydrobromide (6-OHDA, Sigma, 2 × 50 mg/kg, i.p. on day 1, followed by 2 × 100 mg/kg, i.p. on day 4, experiment on day 5), 5-hydroxytryptamine creatinine sulphate (5-HT, Sigma), indomethacin (Sigma), isoprenaline sulphate (Sigma), leucine-enkephalin (Wellcome), levorphanol tartrate (Roche), lysergic acid diethylamide (LSD, Sandoz), methionine enkephalin (Wellcome), morphine hydrochloride (Macarthy), naloxone hydrochloride (Endo), noradrenaline bitartrate (NA, Koch-Light), phentolamine mesylate (Ciba), procaine hydrochloride (Macarthy), propranolol hydrochloride (ICI), reserpine (Koch-Light, 0.5 mg/kg, i.p. daily for 5 days, experiment on day 6, reserpine was dissolved in 0.25% w/v ascorbic acid), tetrodotoxin (Sankyo), theophylline hydrochloride (Light), tolazoline hydrochloride (Ciba), trichloroethylene (Trilene, ICI), (+)-tubocurarine (Tc, Burroughs Wellcome) and tyramine hydrochloride (Sigma).

Results

Responses of the colon in the pithed rat

Effects of morphine on intraluminal pressure within the colon of the pithed rat Normally, the resting intraluminal pressure within the colon of the pithed rat was 5 to 10 mmHg and varied little. Morphine (5 to 10 mg/kg, i.v.) produced regular fluctuations in the intraluminal pressure, which rose above and returned to control levels at approximately minute intervals, following morphine administration (Figure 1). This effect of morphine was reversed by naloxone (10 mg/kg, i.v.) (Figure 1a).

Morphine-induced waves in intracolonic pressure were normally well maintained but occasionally, gradually decreased in amplitude or frequency. The rhythmic fluctuations in pressure were unaffected by hexamethonium (1 mg/kg, i.v.) and were only transiently reduced in amplitude by atropine (1 mg/kg, i.v.), adenosine (2 mg/kg, i.v.) and ATP (2 mg/kg, i.v.) (Figure 1b).

Effects of tubocurarine and hexamethonium on intraluminal pressure within the colon of the pithed rat Two drugs unrelated to morphine also produced rhythmic fluctuations in intraluminal pressure in the colon. These were Tc (5 mg/kg, i.v.) and C_6 (5 mg/kg, i.v.), both of which have nicotinic receptor blocking activity.

Effects of morphine and opioid peptides on responses of the colon to electrical stimulation of the spinal motor

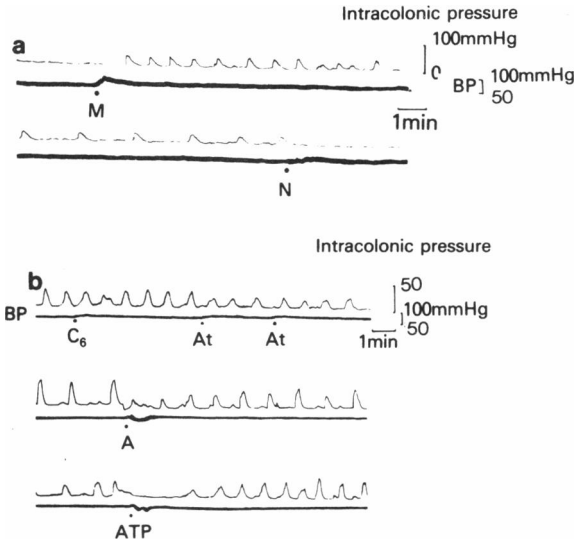

Figure 1 (a) Effect of morphine (M) on intracolonic pressure (upper trace) and blood pressure (lower trace) in the pithed rat. Morphine (10 mg/kg, i.v.) produced regular fluctuations in intra-colonic pressure. This effect was reversed by naloxone (N, 10 mg/kg, i.v.). The lower pair of traces are a continuation of the upper traces. (b) Effect of drugs on morphine-induced fluctuations in intra-colonic pressure (upper trace). The lower trace of each pair is a record of the blood pressure. Morphine-induced waves were unaffected by hexamethonium (C_6, 1 mg/kg, i.v.) and only transiently reduced in amplitude by atropine (At, 1 mg/kg, i.v.), adenosine (A, 2 mg/kg, i.v.) or ATP (2 mg/kg, i.v.). The middle and bottom pairs of traces are continuations of the top traces.

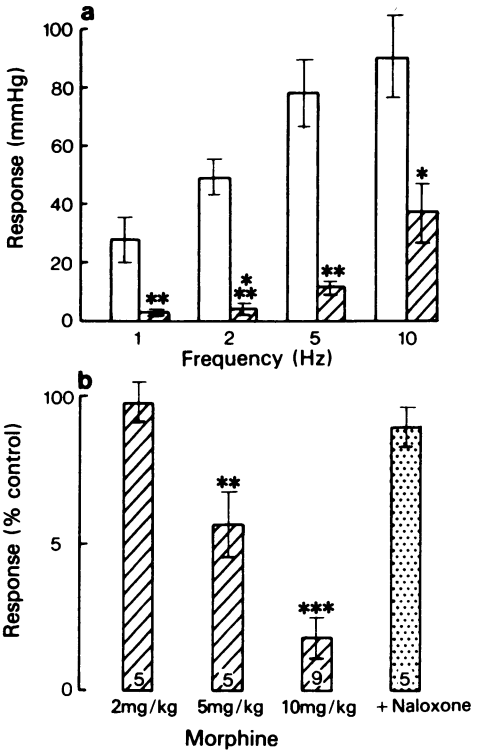

Figure 2 (a) Histogram (open columns) showing increases in intracolonic pressure (means, vertical lines show s.e. mean, $n = 4$) in the pithed rat to electrical stimulation of the sacral parasympathetic outflow with supramaximal voltage pulses of 1 ms duration at different frequencies. Also showing inhibition of these responses by morphine (10 mg/kg, i.v., hatched columns, $n = 4$). Morphine was more effective at low frequencies of stimulation. (b) Histogram (hatched columns) showing dose-related inhibition by morphine (10 mg/kg, i.v.) of the excitatory responses (expressed as percentages of the control; means, vertical lines show s.e. mean) of the rat colon to electrical stimulation of the sacral parasympathetic outflow with supramaximal voltage pulses of 1 ms duration at 10 Hz. Also showing reversal of this inhibition by naloxone (10 mg/kg, i.v., shaded column). The figures in the columns refer to numbers of observations; * $0.05 > P > 0.01$; ** $0.01 > P > 0.001$; *** $P < 0.001$.

outflows in the pithed rat Stimulation of the spinal parasympathetic motor outflows at the level of sacral segments 1 and 2 increased the intracolonic pressure. The optimum frequency of stimulation was 10 Hz. Morphine (5 and 10 mg/kg, i.v.) reduced the response of the colon to stimulation at 10 Hz in a dose-dependent manner and this inhibition was reversed by naloxone (10 mg/kg, i.v.) (Figure 2). Comparison of the effect of morphine (10 mg/kg, i.v.) on the response of the colon to different frequencies of stimulation revealed that morphine was more effective at low frequencies of stimulation, responses to 1 and 2 Hz being almost abolished, while responses to 10 Hz were reduced by only 60% (Figure 2).

Leucine- or methionine-enkephalin (8 μg/kg, i.v.) briefly inhibited the responses of the colon to electrical stimulation at 10 Hz. The transient inhibitory action of the enkephalins may reflect their rapid enzymatic degradation (Hughes, 1975; Hambrook, Morgan, Rance & Smith, 1976). The inhibitory effect of the enkephalins was, like that of morphine, dose-dependent and reversed by naloxone.

Responses of the isolated colon of the rat

Effects of morphine and related drugs on the motility and electrical activity of the rat colon The addition of morphine (10^{-5} M) to the isolated colon caused it to

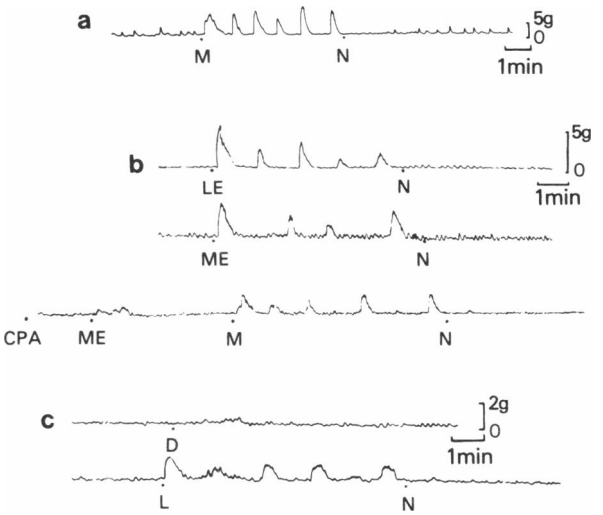

Figure 3 (a) Response of the isolated colon to morphine (M, 10^{-5} M) and inhibition of this response by naloxone (N, 5×10^{-6} M). (b) Effect of leucine-enkephalin (LE, 2×10^{-8} M) and methionine-enkephalin (ME, 2×10^{-9} M) on the motility of the isolated colon. Both peptides produced rhythmic contractions, which were similar to those produced by morphine and were inhibited by naloxone (N, 10^{-7} M). Preincubation of the isolated colon with carboxypeptidase A (CPA, 4 units/ml) prevented methionine-enkephalin (ME, 10^{-8} M) producing rhythmic contractions, which could still be produced by morphine (M, 10^{-5} M). These three traces were obtained in different experiments from three separate preparations. None of the traces is a continuation of the trace in (a). (c) Effects of the stereoisomers, dextrorphan (D, 5×10^{-6} M) and levorphanol (L, 5×10^{-6} M) on the motility of the isolated colon. The active stereoisomer, levorphanol, (L, 5×10^{-6} M) produced rhythmic contractions similar to those produced by morphine, whereas the inactive stereoisomer, dextrorphan (D, 5×10^{-6} M) did not. The response to levorphanol was reversed by naloxone (N, 10^{-6} M). These two traces were obtained in different experiments from two separate preparations. Neither of the traces is a continuation of any of the foregoing traces.

contract immediately and then relax to its initial resting tension. This first contraction–relaxation cycle lasted 1.5 ± 0.1 min ($n = 15$), and was followed at similar intervals by waves of contractions which usually gradually diminished in amplitude (Figure 3a). These contractions were accompanied by bursts of electrical activity, which just preceded each increase in tension. When the colon had ceased responding to the first dose of morphine, a second dose was generally ineffective in causing further contractions. The opiate antagonist, naloxone (5×10^{-6} M), reversed morphine-induced contractions (Figure 3a) and, if administered before morphine, prevented them occurring. The opioid peptides (2×10^{-8} M, 2×10^{-9} M) produced responses similar to that produced by morphine but they were more potent (Figure 3b). This effect of the enkephalins was reversed by naloxone (10^{-7} M) and prevented from occurring by carboxypeptidase A (4 units/ml), which inactivates the enkephalins. Since an important criterion for a response being mediated via specific opiate receptors is that it should exhibit stereospecificity, the effects of the opiate stereoisomers, dextrorphan and levorphanol were examined. The inactive enantiomer, dextrorphan (5×10^{-6} M), had no effect in the colon but the active enantiomer, levorphanol (5×10^{-6} M), produced characteristic contractile activity, which was reversed by naloxone (10^{-6} M) (Figure 3c). In some tissues, spontaneous rhythmic contractile activity occurred even in the absence of morphine or any other opiate, and in others, morphine was ineffective in producing its characteristic response. A chi-square test was used to determine whether there was any association between the increased frequency of occurrence of the contractile activity and the presence of an opiate in the organ bath. This analysis, performed using the data in Table 1, indicated that opiates significantly increased the frequency of occurrence of rhythmic contractile activity in the rat colon.

Analysis of the mechanism of opiate-induced contractions in the isolated colon

Comparison of the effects of morphine and other agonists on the motility of the isolated colon Morphine-induced contractions in the colon were unlike those produced by ACh (Figure 4a), which caused the colon to contract and to remain contracted for several minutes, during which the colon slowly relaxed to its initial resting tension, although the agonist remained in contact with it. Carbachol had a similar effect.

Morphine-induced contractions were also unlike those produced by 5-HT, which caused the colon to contract and either to remain contracted, as did ACh, or to contract and relax irregularly and briefly for 2 to 3 min, after which the colon relaxed to its initial resting tension, despite the continued presence of 5-HT (Figure 4).

In some experiments the colon was exposed to 5-HT (10^{-5} M) continuously for 1 h, at the end of which it was unresponsive to 5-HT (3×10^{-5} M). In such a 5-HT-desensitized colon, morphine (10^{-5} M) still produced its characteristic rhythmic contractile activity (Figure 4b).

The effects of drugs on opiate-induced contractions in the rat colon Opiate-induced contractions were not inhibited even by high concentrations (10^{-4} M) of

atropine, C_6 and Tc. Indeed, these drugs usually increased the size of contractions produced by morphine and in the absence of morphine, caused similar waves of contractions, which were unaffected by naloxone (10^{-5} M) (Figure 5).

Morphine-induced contractions were not inhibited by the 5-HT antagonist, LSD, which occasionally potentiated the amplitude of these contractions. In the absence of morphine, LSD (3×10^{-7} M to 10^{-5} M) also produced rhythmic contractions, which were similar to those produced by morphine but were unaffected by naloxone (10^{-5} M). On the other hand, cyproheptadine (3×10^{-5} M) decreased the amplitude, increased the frequency and eventually, abolished the morphine-induced contractions (Figure 6). Cyproheptadine had a similar effect on LSD-induced contractions and also antagonized the effects of carbachol and KCl (Figure 6).

The amplitude of morphine-induced contractions was potentiated by the α-adrenoceptor blocking drug, phentolamine (10^{-5} M) (Figure 7) but not by the α-adrenoceptor antagonists, ergotamine (10^{-5} M) or tolazoline (10^{-5} M) nor by the β-adrenoceptor antagonist, propranolol (10^{-5} M) (Figure 7). Morphine-induced contractions were also potentiated by the adrenergic neurone blocking drug, guanethidine (10^{-5} M) which occasionally caused contractions similar to those produced by morphine. In contrast, morphine-induced contractions were inhibited in a dose-dependent manner by the catecholamines, adrenaline, isoprenaline and noradrenaline and by the indirectly-acting sympathomimetic, tyramine (3×10^{-5} M) (Figure 7). Tyramine did not inhibit morphine-induced contractions in segments of colon from rats pretreated either with reserpine (Figure 7) or 6-OHDA.

The amplitude of morphine-induced contractions was reduced in a dose-dependent manner by adenosine and the adenine nucleotides AMP, ATP (10^{-5} M) and cyclic AMP, and also by the phosphodiesterase inhibitors, caffeine (10^{-4} M) and theophylline (10^{-4} M).

Morphine-induced contractions were not inhibited by tetrodotoxin (0.3 μg/ml), which potentiated the excitatory effects of morphine and caused contractions similar to those produced by morphine (Figure 8). Tetrodotoxin-induced contractions were not inhibited by naloxone. The local anaesthetic, procaine, did not inhibit morphine-induced contractions and, in high concentrations, caused contractions similar to those produced by morphine (Figure 8).

The responses of the rat colon to field stimulation and the effects of drugs on these responses Field stimulation of the rat colon with supramaximal voltage pulses of 0.2 ms duration for periods of up to 15 s produced complex responses, which varied according to the frequency of stimulation. Low frequencies (0.5 to 5 Hz) produced either no response or a small inhibitory response during stimulation, whereas, stimulation at higher frequencies (10 to 50 Hz) produced a contraction during stimulation. At all frequencies, on cessation of stimulation, there was a brief after-contraction, which diminished in amplitude at higher frequencies and was abolished by indomethacin.

Morphine (10^{-5} M) inhibited the contractile response produced by field stimulation at frequencies between 5 and 50 Hz, the responses obtained at 5 and 10 Hz being reduced to a greater extent than those obtained at 20 and 50 Hz. This inhibitory effect of morphine was overshadowed, especially at low frequencies of stimulation by the large post-stimulus contraction, which was usually enhanced by morphine.

The contractile-response to field stimulation at frequencies above 10 Hz was inhibited by atropine (5×10^{-6} M), after which low frequency stimulation, below 10 Hz, produced a small inhibitory response, which was not inhibited by guanethidine (2×10^{-5} M).

The effects of field stimulation on morphine-induced responses of the rat colon When the excitatory responses to electrical field stimulation were blocked by atropine (10^{-5} M), morphine still caused the colon to

Table 1 The incidence of contractions in rat isolated colon in the absence and presence of opiates

	Control (absence of opiates)	*In presence of morphine*	*In presence of enkephalin*	*Total*
Contractions	5	14	13	32
No contractions	25	2	1	28
Total	30	16	14	60

Results were analysed by the chi-square test, $P < 0.001$. The characteristics of morphine-induced contractions were: mean cycle time (±s.e. mean) = 1.48 ± 0.11 min ($n = 15$); mean amplitude of contraction (±s.e. mean) = 3.90 ± 0.33 g ($n = 15$).

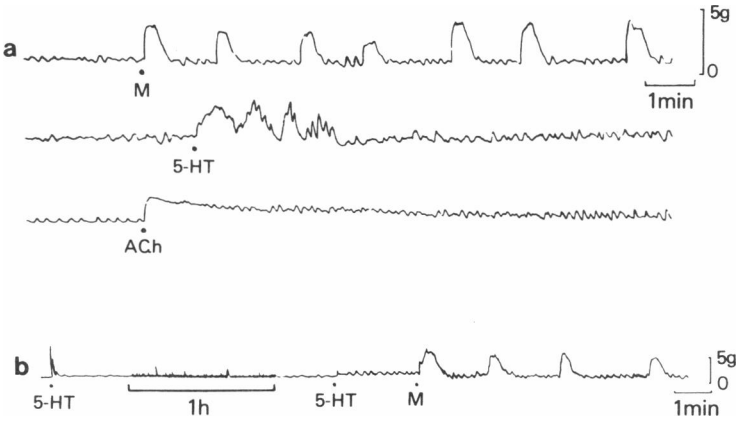

Figure 4 (a) Comparison in three separate preparations of the responses of the isolated colon to morphine (M, 10^{-5} M), 5-hydroxytryptamine (5-HT, 10^{-6} M) and acetylcholine (ACh, 10^{-6} M). (b) Effect of exposing the isolated colon to 5-HT (10^{-5} M) for 1 h. This treatment made the colon unresponsive to 5-HT (3×10^{-5} M) but did not affect its response to morphine (M, 10^{-5} M).

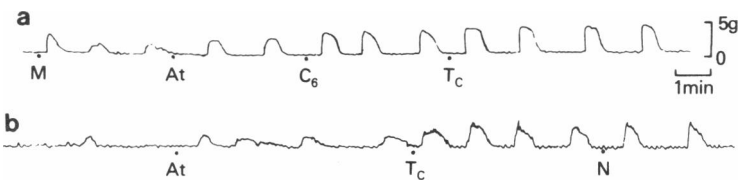

Figure 5 (a) Effects of cholinoceptor antagonists on morphine-induced contractions in the isolated colon. Contractions produced by morphine (M, 10^{-5} M) (upper trace) were not inhibited by atropine (At, 10^{-4} M), hexamethonium (C_6, 10^{-4} M) or (+)-tubocurarine (Tc, 10^{-4}M). These drugs increased the amplitude of contractions produced by morphine. In a separate experiment (b) contractions appeared in the presence of atropine (At, 10^{-4} M). These were potentiated by Tc (5×10^{-5} M) and unaffected by naloxone (N, 10^{-5} M).

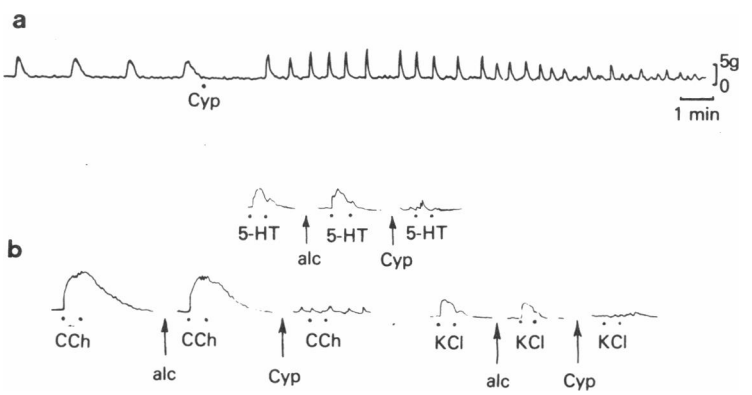

Figure 6 (a) Effects of cyproheptadine (Cyp, 3×10^{-5} M) on morphine-induced contractions in isolated colon. Cyproheptadine increased the frequency, reduced the duration and decreased the amplitude of morphine-induced contractions. The chart speed was constant and morphine (10^{-5} M) was present throughout this experiment. (b) Effects of cyproheptadine (Cyp, 3×10^{-5} M) on responses of the colon to 5-hydroxytryptamine (5-HT, 2×10^{-5} M), carbachol (Cch, 5×10^{-6} M) and KCl (30 mM). Each dose of agonist was applied at the first dot and washed from the bath at the second dot. Cyproheptadine (Cyp, 3×10^{-5} M) inhibited the responses to 5-HT (2×10^{-5} M), carbachol (Cch, 5×10^{-6} M) and KCl (30 mM). Responses of the colon to these agonists were unaffected by ethanol (alc, 0.1 ml), which was the vehicle for cyproheptadine.

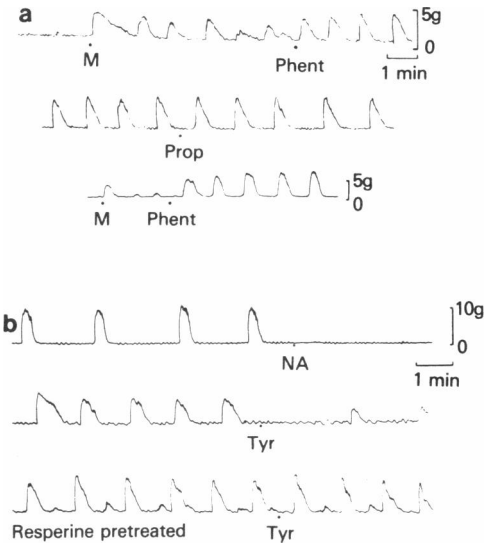

Figure 7 (a) Effects of α- and β-adrenoceptor antagonist drugs on morphine-induced contractions in the isolated colon. Phentolamine (Phent, 10^{-5} M) did not inhibit contractions induced by morphine (M, 10^{-5} M) and, when responses of the colon to morphine had diminished, phentolamine (Phent, 10^{-5} M) increased the amplitude of the contractions. Propranolol (Prop, 10^{-5} M) did not affect morphine-induced contractions. The top and middle traces are a continuous record. The bottom trace is from a separate experiment. (b) Effect of noradrenaline (NA, 5×10^{-6} M) and tyramine (Tyr, 3×10^{-5} M) on morphine-induced contractions in the isolated colon. Morphine-induced contractions were inhibited by noradrenaline (NA, 5×10^{-6} M) and by tyramine (Tyr, 3×10^{-5} M). In reserpine-pretreated tissues, tyramine (Tyr, 3×10^{-5} M) had no effect. The three traces are from separate experiments.

contract rhythmically. In those circumstances, electrical stimulation applied at the peak of a contraction produced an inhibition, which lasted throughout the period of stimulation (Figure 9). This inhibitory effect was unaffected by the adrenoceptor blocking drugs, propranolol (10^{-5} M) or phentolamine (10^{-5} M) (Figure 9), or by guanethidine (2×10^{-5} M). Furthermore, in colon from rats pretreated with reserpine or 6-OHDA, morphine-induced contractions could still be inhibited by field stimulation (Figure 9).

In contrast to the lack of effect of adrenergic neurone or adrenoceptor blocking drugs on the inhibitory response to electrical stimulation, TTX (0.3 μg/ml) abolished the inhibitory responses to field stimulation. TTX also abolished the rebound excitation, which followed field stimulation between the rhythmic contractions induced by a combination of morphine, atropine and guanethidine (Figure 10).

Discussion

The ability of morphine and opioid peptides to inhibit responses of the colon in the pithed rat to electrical stimulation of the motor nerves, and to inhibit responses of the isolated colon to field stimulation, is consistent with previous findings (Paton, 1957). In addition to these inhibitory effects, the opiates also had a direct excitatory effect in the colon. This ability of opiates to cause spasm of smooth muscle has been classified as a non-specific action (Hughes, 1976) and has been attributed to the release by opiates of endogenous 5-HT and ACh (Burks, 1973). The results obtained in this study do not support these views.

In this study, opiates had a characteristic excitatory effect *in situ* in the pithed rat, in which the drug was distributed to the colon via the blood stream, and

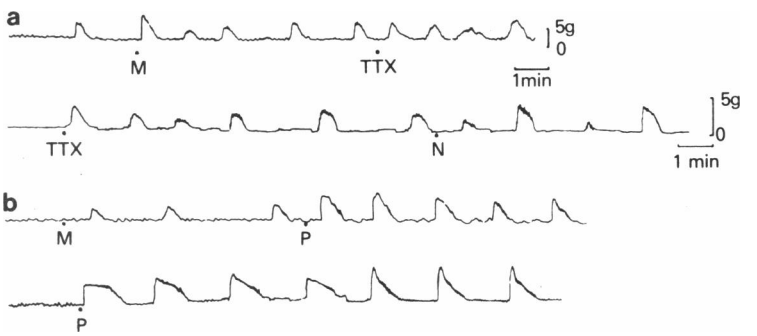

Figure 8 (a) Lack of effect of tetrodotoxin (TTX, 0.3 μg/ml) on contractions induced in the isolated colon by morphine (M, 10^{-5} M). Tetrodotoxin (TTX, 0.3 μg/ml) administered alone (lower trace) produced contractions, which were like those produced by morphine (M, 10^{-5} M) but were unaffected by naloxone (N, 10^{-5} M). The upper and lower traces are not a continuous record and were obtained from different pieces of colon. (b) Procaine (P, 10^{-3} M) potentiated morphine-induced contractions, and when administered alone (lower trace) (P, 10^{-3} M) produced contractions similar to those produced by morphine (M, 10^{-5} M). The upper and lower traces are not a continuous record and were obtained from different pieces of colon.

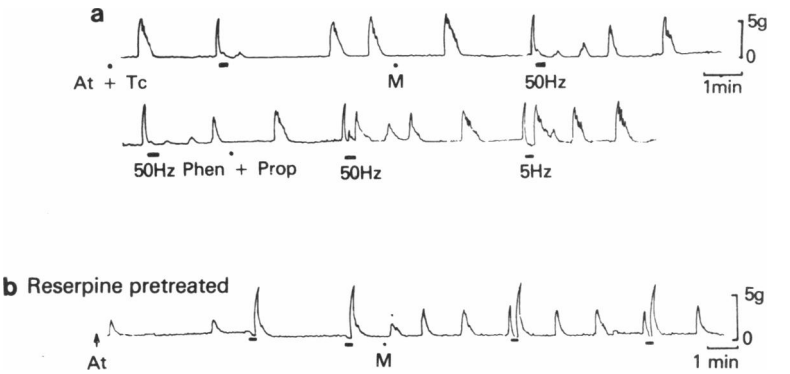

Figure 9 (a) Inhibitory effects of field stimulation on the waves of contraction produced in the isolated colon by atropine (At, 10^{-5} M) and (+)-tubocurarine (Tc, 10^{-5} M). The upper and lower traces are a continuous record from the same preparation. Field stimulation (50 Hz) for the periods indicated by the bars with supramaximal voltage pulses of 0.2 ms duration at the peak of a contraction inhibited the contraction. This inhibition was followed by a small post-stimulus contraction. Morphine (M, 10^{-5} M) did not affect this inhibitory response. After treatment with phentolamine (Phen, 10^{-5} M) and propranolol (Prop, 10^{-5} M), the inhibitory response to field stimulation was still present but was not so well maintained and was followed by a large post-stimulus contraction. Field stimulation at 5 Hz at the peak of a contraction still inhibited the contraction for the duration of the stimulation. (b) Inhibitory effects of field stimulation for the periods indicated by the bars with supramaximal voltage pulses of 0.2 ms duration at 5 Hz in isolated colon from a rat pretreated with reserpine. Atropine (At, 10^{-5} M) produced waves of contraction and blocked the excitatory effects of acetylcholine released by field stimulation at 5 Hz, which produced a slight inhibitory response during stimulation and was followed by a large post-stimulus contraction. Morphine (M, 10^{-5} M) increased the frequency of the waves of contraction and when field stimulation (5 Hz) was applied at the peak of one of these contractions, there was an immediate inhibition for the duration of the stimulation, after which there was a large post-stimulus contraction.

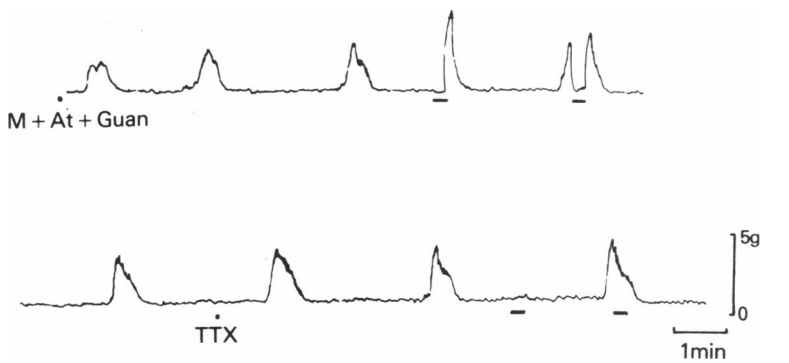

Figure 10 Effect of tetrodotoxin (TTX, 0.3 μg/ml) on responses of the isolated colon to morphine and to field stimulation. The upper and lower traces are continuous. Morphine (M, 10^{-5} M) produced characteristic rhythmic contractile activity. Atropine (At, 5×10^{-6} M) and guanethidine (Guan, 2×10^{-5} M) were added to the bath to block the effects of acetylcholine and the release of noradrenaline by field stimulation, respectively. Thereafter, field stimulation with supramaximal voltage pulses of 0.2 ms duration at 5 Hz for 15 s (indicated by the bars) between morphine-induced contractions, produced little or no response during stimulation, but produced a large post-stimulus contraction. Stimulation at 5 Hz during a morphine-induced contraction produced an immediate and sustained inhibition, followed by a post-stimulus contraction. Tetrodotoxin (TTX, 0.3 μg/ml) did not affect morphine-induced contractions but abolished the post-stimulus contractions following field stimulation, and also inhibited the inhibitory response produced by field stimulation (5 Hz) during a morphine-induced contraction.

also in the isolated colon, exposed to the drug directly in the organ bath. The opioid peptides were more potent than morphine in both preparations but had only a brief excitatory effect in the pithed rat, perhaps because of rapid enzymatic destruction in the blood stream. Although different measures of colonic activity were used in the *in situ* and *in vitro* preparations, the periodicities of the waves of contraction were similar and this similarity suggests a common mechanism of action in these two preparations.

The results indicate that the excitatory action of opiates in the rat colon is a receptor specific effect, since three criteria, which must be satisfied to justify such a conclusion (Lees, Kosterlitz & Waterfield, 1972) have been fulfilled. The first criterion is that the opiate must be effective in low concentrations and the enkephalins fulfil this requirement, although higher concentrations of morphine were required to produce comparable effects. The second is that the responses should be inhibited by specific opiate antagonists. In this study, naloxone readily reversed the effects of morphine but was less effective in reversing the effects of opioid peptides. The third criterion, that the response should be stereospecific, was also satisfied since the active stereoisomer, levorphanol, produced an excitatory effect in the colon, whereas the inactive stereoisomer, dextrorphan, had no such effect, except in high concentrations.

One hypothesis postulated to explain the excitatory action of morphine in the intestine, suggests that morphine produces its effect by releasing 5-HT which acts either on smooth muscle directly or indirectly by releasing ACh from nerves (Burks, 1973). This hypothesis is incompatible with the present results, which show that morphine-induced contractions were unlike those produced by 5-HT or ACh. More conclusive evidence against the involvement of ACh was obtained with atropine, which inhibited standard responses to ACh but did not inhibit the excitatory effects of morphine, and occasionally, even potentiated them.

A similar analysis of the possible participation of 5-HT in the excitatory response to morphine was complicated by the finding that one 5-HT antagonist, LSD, had an excitatory effect like that produced by morphine, and the other, cyproheptadine, was non-specific. However, involvement of 5-HT can be excluded for two reasons. First, because morphine produced excitatory effects in colon rendered insensitive to 5-HT by prolonged exposure to this agonist, and secondly, because morphine still had an excitatory effect in the colon, from which at least neuronal 5-HT had been depleted by pretreatment with reserpine. This evidence conflicts with Burks' hypothesis, postulated to explain the effects of morphine in dog intestine and recently, extended to the rat (Burks, 1976).

Opiates may produce their excitatory effect in the rat colon by a direct action on smooth muscle. Such a hypothesis has been proposed to explain the excitatory effects of atropine (Bortoff & Muller, 1975), local anaesthetics (Vohra, 1970) and tetrodotoxin (Persson, 1971) in various types of smooth muscle. Another explanation of the excitatory effects of opiates could be based on the hypothesis which suggests that intestinal smooth muscle is normally maintained in a state of inhibition by the continuous release of an inhibitory transmitter and that removal of this influence by drugs, unmasks the inherent rhythmic myogenic activity of the muscle (Wood, 1972). The results obtained in this study are consistent with such a hypothesis, since not only did opiates cause rhythmic contractions but other drugs, including the local anaesthetic, procaine, and tetrodotoxin, which specifically interferes with membrane sodium conductance channels in nerve (Narahashi, 1972), produced similar effects. Furthermore, the nicotinic receptor antagonists, C_6 and Tc, produced excitatory effects, similar to that produced by opiates, and, like tetrodotoxin, potentiated the excitatory effects of opiates. The ability of these diverse drugs to interfere with neural processes, albeit by different mechanisms, and to produce effects similar to those produced by opiates, suggests that opiates too may produce their excitatory effect by inhibiting an inhibitory neural mechanism. Evidence for the existence of such a mechanism came from field stimulation experiments. Thus, when the predominant cholinergic motor response of the colon to field stimulation was blocked by atropine, morphine still produced waves of contractions, which could be inhibited by field stimulation. This inhibitory response was probably neurally mediated since narrow pulse widths (0.2 ms) were required to elicit it and since tetrodotoxin or procaine abolished both the inhibitory response and the rebound contraction following field stimulation. This rebound contraction was sometimes the only detectable response to field stimulation and may have arisen from activation of non-adrenergic inhibitory nerves (Bennett, 1966; Furness, 1971). The ability of indomethacin to block the rebound contraction is consistent with such a view (Burnstock, Cocks, Paddle & Staszewska-Barczak, 1975).

The nature of this hypothetical inhibitory mechanism is unknown but is unlikely to be adrenergic since although catecholamines inhibited opiate-induced contractions, and phentolamine and guanethidine potentiated them, other α-adrenoreceptor antagonists, tolazoline and ergotamine, had no such effect. Furthermore, the optimum frequency of stimulation for the inhibitory response was lower than is optimal for stimulating adrenergic nerves. In addition, these inhibitory responses were unaffected by adrenergic neurone or adrenoceptor blocking drugs, and could

still be elicited in colon from animals pretreated with reserpine or 6-OHDA.

Another possibility is that the tonic inhibitory neuronal influence, which normally suppresses myogenic activity in the rat colon, could be provided by non-adrenergic, non-cholinergic inhibitory nerves (Burnstock, 1975). The ability of adenine nucleotides and phosphodiesterase inhibitors to antagonize the excitatory effects of opiates in the colon, is compatible with such a view. However, although the action of opiates on transmitter release at adrenergic and cholinergic neuroeffector junctions has been extensively studied, less is known about their effects at other sites. However, morphine has been shown to depress the non-adrenergic inhibitory responses of guinea-pig taenia coli to transmural stimulation (Shimo & Ishii, 1978). In the present study, morphine may have blocked the release of inhibitory transmitter by a direct action on nerve terminals of inhibitory nerves but this seems unlikely, since morphine-induced contractions could be abolished by field stimulation. This discrepancy can be explained if opiates act proximally to the site of electrical stimulation. A similar hypothesis has been postulated to explain the inhibition by transmural stimulation of atropine-induced contractions in cat intestine (Wood, 1972). Such an explanation requires that opiates block ganglionic transmission perhaps by inhibiting ACh release from a spontaneously active cholinergic neurone which activates the inhibitory neurone. This is supported by observations that opiates depress enteric ganglionic transmission (Dingledine, Goldstein & Kendig, 1974; Ehrenpreis, Sato, Takayanagi, Comaty & Takagi, 1976) and have effects additive with those of atropine, C_6 and Tc on postganglionic cholinoceptors (Wood, Rose & Jackson, 1976).

The results of this study may now be summarised. Opiates and several other drugs produce rhythmic contractile activity in the rat terminal colon *in situ* and *in vitro*. An analysis of this response with drugs and field stimulation provided evidence that the rat colon receives in addition to a motor cholinergic and perhaps an indirect inhibitory adrenergic innervation, a non-adrenergic, non-cholinergic inhibitory innervation. The results suggest that opiates do not produce their excitatory effect in the rat colon by releasing ACh or 5-HT but act via a receptor-specific effect to inhibit the tonic inhibitory influence of non-cholinergic, non-adrenergic nerves on the myogenic activity of the colon. Furthermore, since the inhibitory response to field stimulation could be obtained in the presence of morphine, it appears likely that if opiates do remove an inhibitory neural influence, they do not act directly on the inhibitory neurone but act presynaptically, perhaps to inhibit transmitter release from a cholinergic nerve, which normally activates the inhibitory nerve. Another possibility, which cannot be excluded in this study, is that opiates act directly on smooth muscle and have effects additive with those of procaine and tetrodotoxin, which stimulate the muscle by removing a tonic inhibitory neural influence.

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