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Excitatory Effect of Morphine and Opioid Peptides in the Rat Isolated Colon

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Abstract—Morphine and the opioid peptides cause isolated segments of rat colon to contract and relax rhythmically. This study re-examines two hypotheses to explain this phenomenon: Release of 5-hydroxytryptamine (5-HT)/acetylcholine by morphine or inhibition of a tonically active non-adrenergic, non-cholinergic (NANC) inhibitory mechanism. Rhythmic contractions induced by morphine (5×10^{-6} M) were naloxone sensitive (10^{-6} M) but unaffected by methysergide (10^{-6} M), atropine (10^{-6} M) or pretreatment of rats with *p*-chlorophenylalanine (200 mg kg^{-1} i.p. for four days) which lowered the 5-HT level in the colon from $3{\cdot}73 \pm 0{\cdot}83$ mg g^{-1} in controls to $0{\cdot}41 \pm 0{\cdot}06$ mg g^{-1} ($P < 0{\cdot}001$). The pattern of rhythmic contractions produced by morphine was unlike those produced by 5-HT (5×10^{-6} M), acetylcholine (5×10^{-6} M) or potassium chloride (30 mM). Tetrodotoxin (10^{-6} M), apamin (10^{-8} M), clonidine (2×10^{-8} M), phentolamine (10^{-5} M) or oxprenolol (10^{-5} M) caused rhythmic contractions which were unaffected by naloxone. Clonidine contractions were inhibited by yohimbine (10^{-7} M) but not by prazosin (10^{-6} M). Electrical field stimulation at the peak of a contraction induced by morphine, apamin or clonidine, produced an inhibitory response which was unaffected by atropine, phentolamine, propranolol and guanethidine (all 10^{-5} M). It persisted in colon segments from rats pretreated with reserpine or 6-hydroxydopamine. These results suggest that neither the 5-HT/acetylcholine hypothesis nor inhibition of the NANC mechanism adequately explains the excitatory effect of morphine in the rat colon.

Rat isolated colon longitudinal muscle responds to morphine and opioid agonists with an immediate but transient tonic contraction followed by rhythmic waves of contraction and relaxation (Burks 1976; Gillan & Pollock 1980; Nijkamp & Van Ree 1980; Scheurer et al 1981). While the immediate tonic contraction is believed to reflect the direct contractile effect of morphine and opioid agonists on circular smooth muscle cells (Bitar & Makhlouf 1982), there are two hypotheses to explain the rhythmic waves of contraction and relaxation of the longitudinal muscle. The first suggests that morphine has an excitatory effect because it releases 5-hydroxytryptamine (5-HT) which causes muscle contraction both directly and indirectly through acetylcholine (ACh) release (Burks 1976). The second hypothesis suggests that opioids depress an inhibitory non-adrenergic non-cholinergic (NANC) neural input to circular muscle which normally masks myogenic activity (Gillan & Pollock 1980). Thus opioids acting presynaptically to reduce the release of NANC transmitter might allow the spread of slow waves from the circular muscle to the longitudinal muscle resulting in myogenic activity (Caprilli et al 1982).

Weaknesses exist in these hypotheses, e.g. the 5-HT/ACh hypothesis is contradicted by evidence that morphine contracts the colon in the presence of an ACh antagonist and in tissue rendered unresponsive to 5-HT (Gillan & Pollock 1980).

The purpose of this study was to re-examine these hypotheses. The 5-HT/ACh hypothesis was examined by the use of 5-HT and ACh antagonists, 5-HT depletion or desensitization. The NANC hypothesis was tested by using neuronal blockers.

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Materials and Methods

Responses of rat isolated colon

Male Wistar rats (230–300 g) were stunned and killed by bleeding. From each rat, one 3–4 cm length of terminal colon was excised, emptied of contents and suspended in an organ bath containing 20 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, $NaHCO_3$ 2·5 and glucose 11·1), maintained at 37°C and gassed continuously with O_2/CO_2 (95:5). The lower end of the colon was anchored to the hook of a ring electrode, and the upper end attached by a thread to a Grass FT03 force-displacement transducer mounted vertically above the organ bath. The initial resting force of 2 g applied to each tissue gradually fell to 1 g during the 30 min equilibration period, throughout which the Krebs solution was changed at 15 min intervals. The isometrical responses were displayed on a Grass Polygraph.

Field stimulation of the isolated colon

Segments of colon, suspended in an organ bath containing Krebs solution (37°C) were stimulated electrically through a silver ring electrode with square wave pulses of supramaximally effective voltage, 0·5 ms duration, 1–50 Hz (Palmer or Grass S88 stimulator).

Assay of 5-hydroxytryptamine

The 5-HT content of the colon was assayed flurometrically using *o*-phthalaldehyde (OPT), which forms a fluorescent complex with 5-HT (Curzon et al 1981). Controls and *p*-chlorophenylalanine (PCPA)-treated rats were stunned and

killed by bleeding. From each rat, a segment of terminal colon was dissected free of mesentery and blood vessels, weighed and transferred to cold Krebs solution. Each segment was homogenized in 5 mL of acidified butanol (850 μL HCl in 1 L butanol, 0°C) in a glass tube by a motor-driven Teflon pestle. The volume of each homogenate was adjusted to 25 mL and the homogenate centrifuged (3000 *g*, 10 min, 4°C).

The 5-HT content of each supernatant was determined by a slightly modified version of the method of Curzon & Green (1970) necessary for the preparation of a tissue blank. It was made by adding 10 μL of potassium ferricyanide (0·2% w/v) to an aliquot of the supernatant. This procedure oxidizes all of the 5-HT present (Andén & Magnusson 1967).

A 2 mL aliquot of each supernatant was transferred to a tube containing 5 mL of n-heptane and 600 μL of acidified cysteine solution (1% w/v in 0·1 M HCl). The contents of each tube were mixed for 2 min and centrifuged (3000 *g*, 5 min, 4°C).

The upper organic phase was discarded, and a 200 μL aliquot of the aqueous phase was incubated for 15 min at 77°C with 20 μL 1% cysteine and 800 μL HCl containing 0·004% *o*-phthalaldehyde. The fluorescence was measured, when the tubes had cooled to room temperature (Aminco-Bowman spectrophotofluorimeter, activation wavelength 370 nm, emission wavelength 480 nm).

Standards were prepared by dissolving 5-HT in distilled water and 200 μL, containing from 50 to 200 ng 5-HT was added to tissue extracts to serve as internal standard. These were carried through the entire assay procedure.

Animal pretreatment schedules

Reserpine was dissolved in glacial acetic acid (0·2 mL) and diluted with water. Rats received 2 mg kg^{-1}, i.p. daily for 4 days in 0·2 mL/100 g. Control rats received an equivalent volume of appropriately diluted acetic acid. The 6-hydroxydopamine (6-OHDA) solution was adjusted to give 0·2 mL of solution/100 g. Rats received 2×50 mg kg^{-1} i.p. on day 1; 2×100 mg kg^{-1} i.p. on day 5. Controls received saline. The tissues were examined on day 6. PCPA 200 mg kg^{-1} i.p. was given daily for 4 days while the controls received saline. The tissues were examined on day 5.

Analysis of results

All the graphs show the mean ± standard error of the mean (s.e.m.). Student's *t*-test and the Duncan multiple range test were used for statistical analysis.

Drugs

Acetylcholine chloride (Koch-Light); apamin (Sigma); atropine sulphate (BDH); carbamoylcholine chloride (Sigma); clonidine (Boehringer Ingelheim); D-alaglymepheglyol (Sigma); guanethidine monosulphate (Ciba); 6-hydroxydopamine hydrobromide (Sigma); 5-hydroxytryptamine creatinine sulphate (Sigma); leucine enkephalin (Sigma); methionine enkephalin (Sigma); methysergide bimaleate (Sandoz); morphine hydrochloride (Macarthys); naloxone hydrochloride (Winthrop); oxprenolol hydrochloride (Ciba); *p*-chlorophenylalanine methylester (Sigma); phentolamine mesylate (Ciba); prazosin (Sigma); propranolol hydrochloride (ICI); reserpine (Koch-Light); substance P (Sigma); tetrodotoxin (TTX, Sankyo); yohimbine hydrochloride (Koch-Light).

Results

Effects of morphine and of opioid peptides on the motility of the isolated colon

The rat isolated colon showed little spontaneous motor activity. Morphine (10^{-6}–10^{-5} M) contraction usually occurred immediately, but sometimes after a delay of several minutes. The contraction was followed by a relaxation to the base line with morphine still in the bath. This initial contraction was not followed by waves of contraction and relaxation which continued at intervals thereafter (Fig. 1). The frequency of the phasic activity varied between animals and in tissues from the same animal during the course of an experiment. This phasic activity was abolished by naloxone (10^{-6} M) (Fig. 1) but was not affected by methysergide (10^{-6} M) or atropine (10^{-6} M). Phentolamine (10^{-6} M), plus propranolol (10^{-6} M) produced 60 ± 5·0% ($P < 0·05$, n = 5) increase in tension developed to morphine (Fig. 2). DAGO (2×10^{-8} M), leucine enkaphalin (10^{-8} M) or methionine enkephalin (2×10^{-7} M) also caused naloxone-sensitive ($10^{-7} - 2 \times 10^{-7}$ M) contractions (Fig. 1). Naloxone in concentrations up to 10^{-5} M did not affect contractions elicited by ACh, 5-HT or substance P (not shown). The initial contraction to morphine was concentration-related and was competitively antagonized by 30 min preincubation with naloxone (3×10^{-7} M). In contrast, 30 min preincubation with the 5-HT antagonist methysergide (3×10^{-7} M) shifted the dose response curve non-competitively (Fig. 3).

Comparison of the response of the colon to morphine, 5-HT, ACh, and KCl

The pattern of contractions produced by morphine was unlike that to other agonists. KCl (30 mM) produced a biphasic contractile response with an initial transient phase followed by a secondary sustained phase. ACh (5×10^{-6} M) elicited a partially maintained tonic contraction. 5-HT

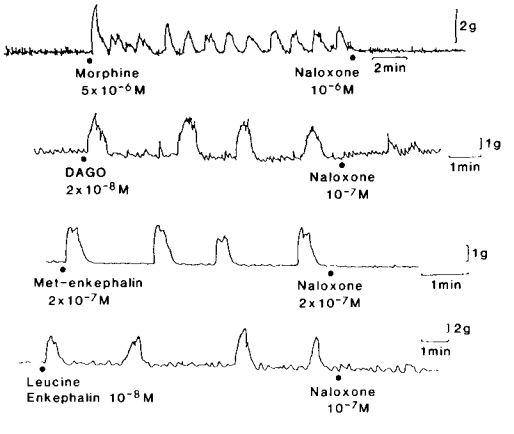

FIG. 1. Excitatory effect of morphine and opioid peptides in the rat colon. Morphine (5×10^{-6} M), D-alaglymepheglyol (DAGO) (2×10^{-8} M), Met-enkephalin (2×10^{-7} M), and Leu-enkephalin (10^{-8} M) all produced naloxone-sensitive rhythmic contractions. Each agonist was added to the organ bath at ● and remained in contact with the tissue until the end of the experiment. Each tracing was from a different experiment.

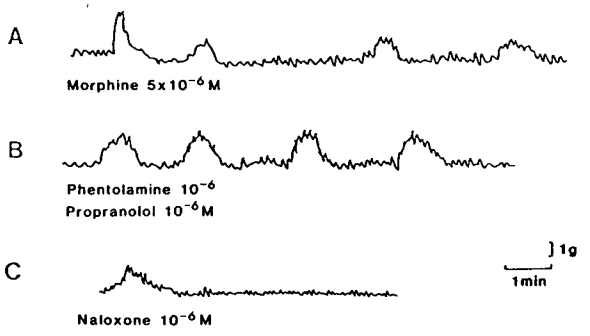

FIG. 2. Effect of adrenergic antagonists on morphine contractions in the isolated colon. Morphine (5×10^{-6} M) produced waves of contractions (A). These were potentiated by propranolol (10^{-6} M) plus phentolamine (10^{-6} M) ($P < 0.05$) (B). Naloxone (10^{-6} M) inhibited the contractions (C). Traces A, B, and C are from the same experiment.

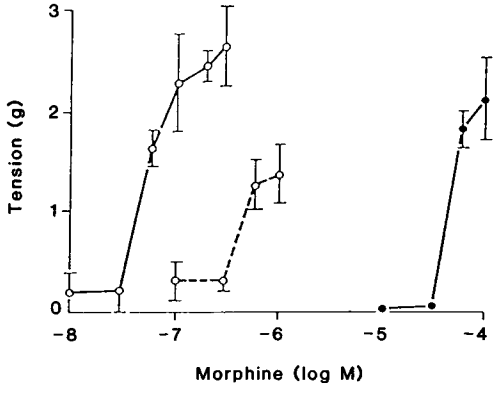

FIG. 3. Effect of methysergide and naloxone on the initial contraction of the rat colon induced by morphine. O——O, control response, O- - - - - -O, response in the presence of methysergide (3×10^{-7} M), ●————●, response in the presence of naloxone (3×10^{-7} M). Values (mean ± s.e.m.) n = 5.

(5×10^{-6} M), briefly caused the colon to contract and relax irregularly after which it became quiescent (Fig. 4).

Effect of PCPA pretreatment or 5-HT autodesensitization on response of the colon to morphine

PCPA reduced the 5-HT content of the colon ($P < 0.001$) (Fig. 5). In colon so treated, morphine (5×10^{-6} M) elicited naloxone (10^{-6} M)-sensitive rhythmic contractions. The PCPA pretreated tissue showed a faster rate of contraction compared with controls.

In Fig. 6, the colon was desensitized to the contractile effect of 5-HT (5×10^{-6} M) by its cumulative addition. The response diminished with each administration until a higher concentration of 5-HT (5×10^{-5} M) produced no response. At this point, morphine (5×10^{-6} M) caused phasic contractions that were inhibited by naloxone (10^{-6} M).

Responses of the colon to adrenoceptor agonists and antagonists

Clonidine (2×10^{-8} M) caused rhythmic contractions that were unaffected by naloxone and prazosin (10^{-6} M), but were inhibited by yohimbine (10^{-7} M) (Fig. 7). Phentolamine, propranolol or oxprenolol (10^{-5} M), but not prazosin (10^{-5} M) also produced rhythmic contractions (n = 5).

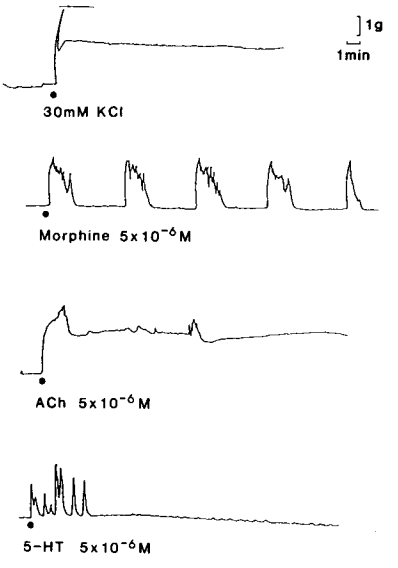

FIG. 4. Comparison of the responses of the rat colon to potassium chloride (KCl, 30 mM), morphine (5×10^{-6} M) acetylcholine (ACh, 5×10^{-6} M) or 5-hydroxytryptamine (5-HT, 5×10^{-6} M). The response of the colon to morphine was unlike the responses to KCl, ACh or 5-HT.

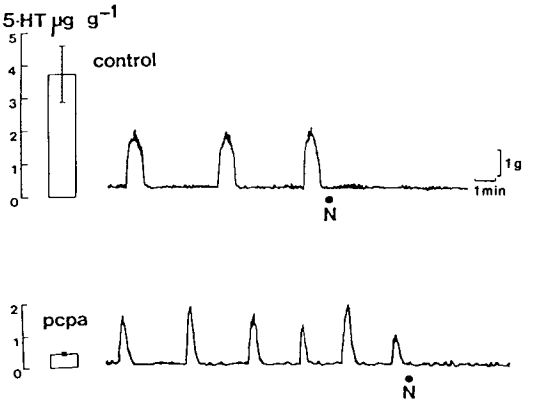

FIG. 5. Excitatory effect of morphine on PCPA-pretreated colon. The upper panel shows the 5-HT content of the control colon and the rhythmic contractions produced by morphine (5×10^{-6} M). The contractions were antagonised by naloxone (10^{-6} M) added at N. The lower panel shows the 5-HT content of a PCPA-pretreated colon and the naloxone (10^{-6} M)-sensitive contractions produced by morphine (5×10^{-6} M) PCPA-pretreated colon showed a faster rate of contraction. Each histogram is a mean of five observations. The I-bars represent standard error of the mean. $P < 0.001$ compared with control.

Response of the colon to apamin or TTX

Apamin (10^{-8} M) or TTX (10^{-6} M) produced rhythmic contractions which continued for as long as the drug remained in contact with the tissue (Fig. 8). Naloxone (5×10^{-6} M), atropine (10^{-6} M), or methysergide (10^{-6} M) did not affect contractions produced by apamin or TTX.

Response of the rat colon to electrical field stimulation

Field stimulation of colon segments with supramaximally effective voltage, pulses of 0·5 ms and duration for periods of up to 10 s, produced responses which varied according to the tone of preparation and the frequency of stimulation. Below 5 Hz, there was either no response or an inhibitory or

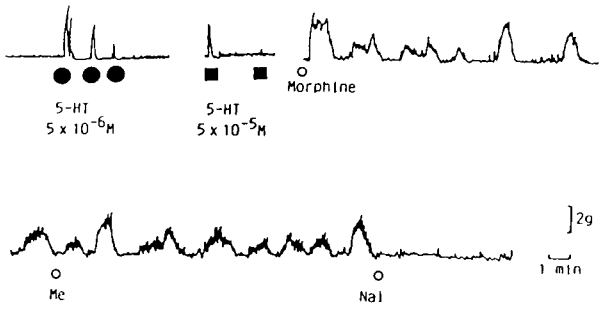

FIG. 6. Excitatory effect of morphine in colon desensitized to 5-hydroxytryptamine (5-HT). Desensitization was produced by cumulative application of increasing concentrations of 5-HT. 5-Hydroxytryptamine (● 5-HT, 5×10^{-6} M) was added three times. A higher concentration of 5-HT (■ 5×10^{-5} M) was again added. The second addition of 5-HT (■ 5×10^{-5} M) did not produce any response. Without washing out the organ bath, addition of morphine (5×10^{-6} M) elicited contractions which persisted in the presence of methysergide (Me, 10^{-5} M) but were inhibited by naloxone (Nal, 10^{-6} M). Both panels are from the same experiment.

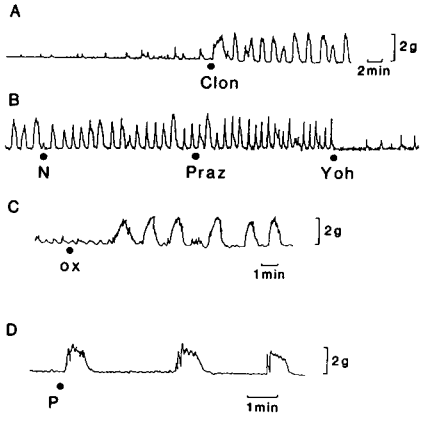

FIG. 7. Excitatory effect of clonidine and adrenoceptor antagonists on the rat colon. Clonidine (Clon, 2×10^{-8} M) produced contractions, which were not inhibited by naloxone (N, 10^{-6} M) and prazosin (Praz, 10^{-6} M) but were inhibited by yohimbine (Yoh, 10^{-7} M). Trace B is a continuation of trace A. Oxprenolol (OX, 10^{-5} M) or phentolamine (P, 10^{-5} M) produced rhythmic contractions in the colon (n = 5). Traces A and B are from the same experiment. Traces C and D are from different ones.

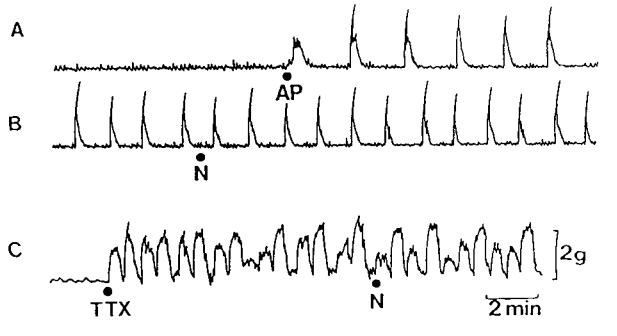

FIG. 8. Excitatory effect of apamin or TTX. Apamin (10^{-8} M) or TTX (10^{-6} M) added at AP in trace A and TTX in trace C elicited rhythmic contractions which were unaffected by naloxone (5×10^{-6} M) added at N in traces B and C. Traces A and B are from the same experiment. Trace C was from a different experiment.

contractile response. Stimulation at frequencies above 5 Hz produced during stimulation a contraction which was not well maintained. Following stimulation, a post-stimulus contraction occurred at all frequencies (Fig. 9) morphine (5×10^{-6} M) or atropine (5×10^{-6} M) (not shown) produced a frequency-dependent reduction of 50–100% of the motor response to electrical stimulation.

Inhibitory response of the rat colon to electrical field stimulation

The resting colon develops little maintained tone on its own, hence the inhibitory response to electrical field stimulation was usually small. When the tone increased during spontaneous rhythmic contractions, electrical field stimulation at the peak of a wave produced an inhibition. Spontaneously generated waves of contraction offered the best opportunity for studying the inhibitory response uncomplicated by the presence of drugs. As the occurrence of these waves was rare, advantage was, therefore, taken of the rhythmic contractions produced by various drugs. In this way, the nature of the inhibitory response was studied.

In the presence of guanethidine, atropine, phentolamine and propranolol (all 10^{-5} M) to block adrenergically- and cholinergically-mediated responses, electrical field stimulation at the peak of a morphine-induced contraction produced a well-maintained inhibition during stimulation with a return to the original (elevated) tone when stimulation ceased (Fig. 10). The optimum frequency for this NANC inhibitory response was less than 5 Hz. The response persisted in tissues pretreated with reserpine or 6-OHDA (not shown), but was reduced by TTX (3×10^{-6} M) (Fig. 10). Stimulation at the peak of an apamin-induced contraction produced a slight inhibitory response, which was converted into a well maintained inhibition during stimulation by atropine (10^{-5} M) (Fig. 11).

Discussion

The findings of this study confirm some of the results obtained by other workers (Gillan & Pollock 1980; Nijkamp & Van Ree 1980; Scheurer et al 1981) that morphine and the opioid peptides produce rhythmic contractile activity in the rat terminal colon. However, they cannot be explained by any previous hypothesis. One of the mechanisms postulated

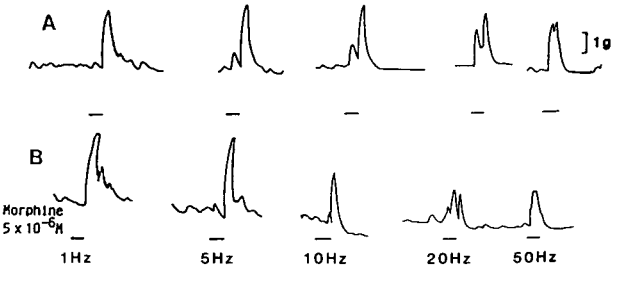

FIG. 9. Effect of morphine on the responses of the rat colon to electrical field stimulation. Trace A shows the responses of the colon to field stimulation in the absence of morphine; during stimulation contractile responses were obtained, followed by post-stimulus contraction. In trace B the presence of morphine (5×10^{-6} M) reduced contractile responses produced during and sometimes after stimulation. Traces A and B are from the same experiment.

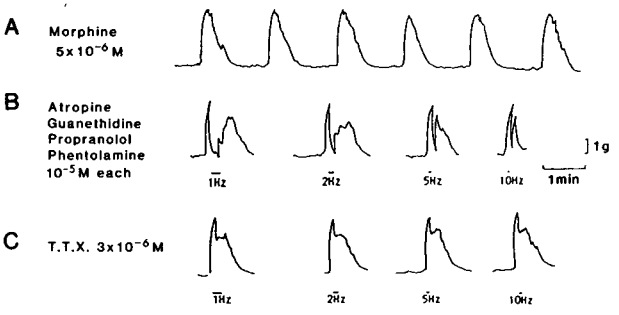

FIG. 10. Non-adrenergic, non-cholinergic inhibitory response of the isolated colon to electrical field stimulation (10 pulses, 0·5 ms, frequency as indicated). In traces B and C inhibitory responses were obtained to electrical field stimulation at the peak of morphine (5×10^{-6} M)-induced contractions in the presence of atropine, guanethidine, propranolol and phentolamine (10^{-5} M) each. In trace C responses were reduced but not prevented by TTX (3×10^{-6} M). Traces A, B, C are a continuation of the same experiment.

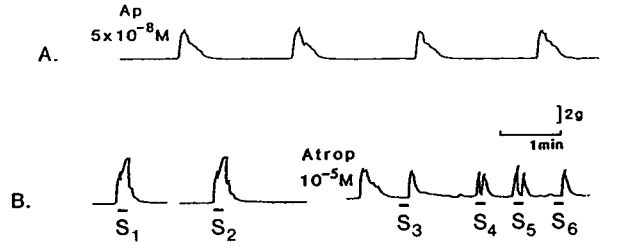

FIG. 11. Effect of apamin on the isolated colon of the rat. Trace A shows rhythmic contractions produced by apamin (5×10^{-8} M). Electrical field stimulation (10 Hz, 0·5 ms for 10 s), at the peak of an apamin-induced contraction (S_1, S_2) produced slight inhibitory response in trace B. The addition of atropine (10^{-5} M) converted the response into a well-maintained inhibition during stimulation (S_4, S_5). Field stimulation between apamin contractions produced during stimulation either no response (S_3) or an inhibitory response (S_6) followed by post stimulus contraction.

involves the release of 5-HT and ACh. From experiments on the small intestine of rat and dog, Burks (1973, 1976) and Huidobro-Toro & Way (1981) suggested that morphine released 5-HT which then stimulated the smooth muscle both directly, and indirectly by releasing ACh from cholinergic nerves.

In the present study, the 5-HT antagonist methysergide non-competitively inhibited the initial contraction to morphine, while it was ineffective against the rhythmic contractions. In contrast, naloxone competitively inhibited the initial response and abolished the phasic activity.

The results obtained with PCPA-pretreated rats, provided evidence against the involvement of 5-HT in the rhythmic activity, since in such colon segments, morphine produced naloxone-sensitive rhythmic contractions. However, since a small residual quantity of 5-HT remained in the colon from PCPA-treated rats, and the rate of contraction is faster in such preparations, the use of PCPA alone does not eliminate the possible involvement of 5-HT. The characteristic excitatory effect of opioids could not be mimicked by administered ACh or 5-HT. In addition, atropine or 5-HT desensitization did not interfere with the morphine effect. Taken together, these results do not support the 5-HT/ACh hypothesis since experimental procedures which modified the 5-HT/ACh level or action in the colon did not abolish morphine activity. Fontaine & Reuse (1985) reached a similar conclusion about the non-involvement of 5-HT/ACh in the excitatory response of the mouse colon to opioids. In contrast, Huidobro-Toro & Way (1981), concluded that the excitatory effect of opioids was mediated by 5-HT/ACh. Those workers pointed out that their Long Evans rats were more sensitive to opioids than other strains such as the Wistar rats we used. However, it is unlikely that this difference could explain the discrepancy between the results obtained in the two studies.

An alternative explanation of the excitatory effect of opioids is that morphine removes a tonic inhibitory (NANC or adrenergic) neural influence and as a consequence unmasks myogenic activity (Gillan & Pollock 1980; Nijkamp & Van Ree 1980; Scheurer et al 1981; Moritoki et al 1984; Fontaine & Reuse 1985). Electrical field stimulation of the colon was used to study this possibility. Field stimulation above 5 Hz produced an excitatory response followed by a post stimulus contraction. This response was antagonized by atropine, TTX or morphine. Since morphine did not affect responses to administered ACh (Cherubini et al 1985), it is likely that morphine acted on presynaptic nerves to inhibit ACh release (Down & Szerb 1980) either by hyperpolarizing the soma of the enteric neurons (Wood 1980) or by blocking conduction in nerve cell processes (Morita & North 1981).

Field stimulation at frequencies below 5 Hz caused either an atropine sensitive contraction or an inhibitory response followed by post-stimulus contraction. The inhibitory response was not abolished by atropine, adrenergic blockers, or by pretreatment with reserpine or 6-OHDA. However, because the rat colon rarely develops and maintains tone on its own, the inhibitory response was further studied by taking advantage of the rhythmic contractions produced by various drugs. Field stimulation applied at the peak of a morphine-induced contraction produced an inhibitory response which was maximal at low frequencies of stimulation and was unaffected by atropine, or drugs blocking adrenergic receptors or neurons indicating its NANC nature. The fact that inhibitory responses to electrical field stimulation were obtained at the peak of morphine-induced contractions suggests that if morphine causes rhythmic contractions by removing a tonic inhibitory neural influence then it is unlikely to be an NANC inhibitory influence, since this NANC response can be more clearly demonstrated when morphine is present and has inhibited ACh release. Previously, when it was suggested that the tonic inhibitory influence removed by morphine might be NANC (Gillan & Pollock 1980), the paradox of the persistence of this inhibitory response at the peak of an opioid-induced contraction was explained by postulating that morphine acted at the ganglionic site preceding the inhibitory nerve. However, in guinea-pig colon and taenia caeci where morphine was postulated to act presynaptically or at the ganglionic site to inhibit NANC transmission, morphine also prevented neurogenic relaxation elicited by electrical field stimulation (Shimo & Ishii 1978; Tonini et al 1985).

Results with apamin which unlike morphine can either act directly on the smooth muscle cells by blocking Ca^{2+}-dependent potassium channels (Banks et al 1979) or presynaptically to inhibit both neurally induced transmitter release and smooth muscle relaxation in guinea-pig taenia caeci and fundic muscle (Grider & Makhlouf 1987), suggest

that rhythmic contractions can be produced in the rat colon with NANC inhibitory transmission remaining unaffected. Field stimulation applied at the peak of an apamin-induced contraction produced only a small inhibition. Atropine converted this inhibitory response into a well-maintained inhibition during stimulation, suggesting that ACh released by field stimulation normally masks the inhibitory response. Thus, it appears that NANC transmission survives both morphine and apamin, and is therefore unlikely to be involved in the waves of rhythmic contractions produced by these drugs. Contreras et al (1988) reached a similar conclusion about the non-involvement of NANC mechanism in the excitatory effect of morphine in the mouse isolated colon.

At the moment, we do not have any direct experimental evidence to support the proposal of Nijkamp & Van Ree (1980) that morphine may be inhibiting an adrenergic mechanism. However, indirect evidence to support the existence of such a mechanism includes the potentiation of morphine contractions by phentolamine plus propranolol, the higher incidence of spontaneous rhythmic contractions in reserpinized colon, the elicitation of rhythmic contractions by the α_2-adrenoceptor agonist clonidine. Furthermore, α_1-adrenoceptor antagonists produced similar contractions. If the opioid-associated contraction is caused by removal of a tonic adrenergic inhibitory mechanism, the inability of adrenergic blockers to antagonize the excitatory opioids effect (Scheurer et al 1981; Moritoki et al 1984; Fontaine & Reuse 1985) is not surprising since such agents would themselves be expected to cause contractions. Their lack of effect does not necessarily preclude an adrenergic-associated mode of action.

In conclusion, these results do not agree with the hypothesis that morphine produces its excitatory effect in the rat colon solely by the release of 5-HT and ACh. These results have also provided evidence inconsistent with the proposition that morphine and other drugs may produce their excitatory action by inhibiting NANC inhibitory mechanism. The role of the adrenergic mechanism in these effects merits further studies.

Acknowledgements

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