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EFFECTS OF OPIOIDS ON NONCHOLINERGIC EXCITATORY RESPONSES OF THE GUINEA-PIG ISOLATED ILEUM: INHIBITION OF RELEASE OF ENTERIC SUBSTANCE P

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1 Experiments were carried out to determine whether opiates and opioid peptides could affect noncholinergic excitatory responses of the isolated guinea-pig ileum.

2 Transmural field stimulation (10–20 Hz) of an atropine pretreated, intact segment of gut produced a contracture that could be elicited repeatedly without significant variation in magnitude.

3 This noncholinergic contracture was significantly reduced 75.3 ± 8.3% (mean ± s.e.mean) by tetrodotoxin (TTX; 1 μg/ml) and by desensitizing the preparation to substance P (76.3 ± 10.1%).

4 Morphine (5×10^{-6} M) as well as the opioid peptides D-Ala^2, N-Phe^4, Met-(0)-01 (FK 33-824; 9×10^{-7} M), D-Met^2-Pro^5 enkephalin (3×10^{-7} M) and D-Ala^2-D-Leu^5-enkephalin (5×10^{-6} M) inhibited the magnitude of the noncholinergic contracture but did not alter contractile responses to exogenous substance P (4×10^{-11} M–4×10^{-10} M).

5 Pretreatment with the nicotinic receptor blocker, hexamethonium (10^{-5}–10^{-4} M) reduced by about 35% the magnitude of the atropine-resistant contracture but did not affect inhibitory responses to morphine or opioid peptides. Thus the inhibition produced by morphine on the 20 Hz contracture does not involve a nicotinic cholinergic mechanism.

6 Naloxone pretreatment (10^{-6} M) in the presence of hexamethonium (10^{-5}–10^{-4} M) enhanced the magnitude of the noncholinergic contracture without affecting responses to exogenous substance P (4×10^{-11}–4×10^{-10} M).

7 These data suggest that substance P is the main, if not the sole, mediator of the atropine-resistant 20 Hz contracture and indicate further that exogenous as well as endogenous opioids can modulate the release of this enteric peptide.

Introduction

The isolated ileum of the guinea-pig displays many of the characteristics of the response of the central nervous system (CNS) to opiates and has been used successfully in the study not only of the acute effects of opiates but also the long term effects of tolerance and dependence (Paton, 1957; Goldstein & Schulz, 1973; Kosterlitz, Lord, & Watt, 1973; Kosterlitz & Waterfield, 1975; Schulz & Herz, 1976; Gintzler, 1979; 1980). Previous studies have shown that there are at least two separate neuronal pathways that mediate naloxone-precipitated enteric withdrawal from opiates. One involves the excitation of post-ganglionic cholinergic neurones, the release of acetylcholine (ACh) and the activation of smooth muscle muscarinic receptors (Schulz & Herz, 1976). The other pathway is resistant to atropine pretreatment and involves the release of enteric 5-hydroxytryptamine (serotonin, 5-HT), which then acts through the enteric nervous system to release substance P (Gintzler, 1979; 1980). Substance P is, thus, the final excitatory transmitter mediating the noncholinergic component of gut withdrawal. The involvement of 5-HT- and substance P-containing neurones in the manifestation of withdrawal suggests that these types of neurone, known to be intrinsic components of the enteric nervous system (Nilsson, Larsson, Hakansson, Brodin, Sundler & Pernow, 1975; Pearse & Polak, 1975; Dreyfus, Bornstein & Gershon, 1977; Dreyfus, Sherman & Gershon, 1977; Schultzberg, Dreyfus, Gershon, Hokfelt, Elde, Nilsson, Said & Goldstein, 1978), might also be able to subserve acute responses to narcotics. This idea is further supported by the observation that the nerve-mediated, atropine-resistant contracture produced by low concentrations of exogenous 5-HT (2.5×10^{-8} M) can be abolished by pretreatment with morphine in a naloxone-reversible fashion (Gintzler, 1979).

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Most studies involving opiates have been done on the longitudinal muscle-myenteric plexus (LM-MP) preparation. It is known that both cholinergic neurones and responses to ACh survive in this preparation. However, it has not been clearly demonstrated that the numerous other neurotransmitter systems known to be present in the gut are maintained as well. Thus, if one uses only the LM-MP preparation, it is hard to tell if a certain component is simply not involved in a given response or if it was eliminated in the process of preparing the LM-MP preparation. In view of these considerations, an intact segment of ileum was used in all of the experiments.

The present paper describes experiments designed to determine the acute effects of morphine and opioid peptides on nerve-mediated, noncholinergic excitatory responses of guinea-pig isolated ileum to transmural field stimulation.

Methods

Male albino guinea-pigs weighing 375–400 g were used. The animals were killed by exsanguination, the terminal portion of the ileum was removed and the last 10 cm were discarded. The lumen of the gut was flushed with 10 ml of Krebs solution, and the preparation (4 cm) was mounted over the short end of a 'J' shaped glass tube which also contained a silver wire that functioned as a stimulating electrode. The mounted preparation was then placed into a 25 ml organ bath which was kept at 37°C. Resting tension was fixed at 1.0 g. The ileum was stimulated by transmural electrical stimulation essentially as described previously (Gintzler, 1979). Rectangular current pulses of 0.2 ms duration and of sufficient strength to produce a maximal response to a single shock were applied to the electrodes once every 10 s. After several minutes of such stimulation, atropine (2 μM) was added to the organ bath and left in contact with the tissue for at least 15 min before starting the experiment; thereafter atropine was added after each wash period. This concentration of atropine was sufficient to block completely the responses elicited by supramaximal field stimulation and by exogenous ACh (10^{-5} M), a concentration that is 50 fold higher than that needed to produce a maximal contracture (Gintzler, 1979). The abolition of contractions in response to electrical stimulation (0.1 Hz) was used to assess the effectiveness of the muscarinic cholinoceptor blockade in each experiment. All subsequent responses of the preparation to various manipulations were elicited while in the presence of the same concentration of atropine (2 μM).

Isometric responses of the gut were recorded with a force displacement transducer connected to a Brush pen recorder. All of the experiments were carried out on pieces of ileum suspended in modified Krebs solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2.2H_2O$ 2.5, $MgCl_2$ 1.2, $NaH_2PO_4.H_2O$ 1.2, $NaHCO_3$ 25 and glucose 11. The buffer was bubbled with a mixture of 95% O_2/5% CO_2 which maintained the pH at 7.4.

The minimum frequency required for repeatedly producing noncholinergic contractures without significant variations in magnitude was found to be 10–20 Hz. Therefore noncholinergic excitatory responses of the guinea-pig ileum were elicited by applying to the electrodes rectangular pulses of the same magnitude and duration as described above but at a frequency of 20 Hz. The stimulation was continued until the magnitude of the resulting contracture was maximal. Following each response, both the serosal and mucosal surfaces were washed three times and the preparation was allowed to rest for 5 min. This cycle was repeated until the magnitude of 3 successive contractures were within 5% of each other. Test drugs were added to the bath at the indicated times during the 5 min rest period and the resulting change in the magnitude of the contracture was calculated by using the mean magnitude of the previous 3 contractures as the control response. The effects of several concentrations of opioid peptides were tested on the same tissue to determine the concentration that produced an inhibition equivalent to that which was produced by morphine (5×10^{-6} M) in the same preparation; in subsequent experiments only a single concentration of peptide was tested. Responses to morphine (5×10^{-6} M) were also determined in each experiment for comparison. The effects of morphine and the opioid peptides were determined twice in each experiment in alternate sequence. The data from each experiment were pooled and the mean values ± s.e.mean were calculated. Responses before and after naloxone pretreatment were analysed by using Student's *t* test (2 tailed). When the magnitude of the substance P-induced contracture was determined, the peptide was left in contact with the preparation only long enough to reach a maximal response. Following each addition of substance P, both the serosal and mucosal surfaces were washed and the preparation was allowed to rest for 5 min before the next addition. At least 4 responses to each concentration of substance P were obtained before determining the magnitude of the response while in the presence of morphine. This cycle and the one involving the 20 Hz contracture was repeated a minimum of 2 times in each experiment. The concentration range over which responses to exogenous substance P were studied was 4×10^{-11} M–4×10^{-10} M. This concentration range was chosen since it produced a range of contracture tension that was comparable to that observed in

response to 20 Hz stimulation. Data obtained were analysed by using Student's t test (2 tailed).

Desensitization to substance P was accomplished by adding the peptide (2×10^{-8} M) to the bath once every 10 min over a 30 min period, the preparation was not washed between additions; contractile responses of desensitized preparations were determined while still in the presence of the added substance P.

The experimental drugs were added to the organ bath in 10–200 μl of distilled water except for substance P which was dissolved in 0.01 M acetic acid. All of the chemicals used to prepare the buffer were of the highest purity obtainable. The following drugs were used: (−)-morphine sulphate (Mallinckrodt), (+)-morphine (National Institute of Health), naloxone hydrochloride (Endo Laboratories), atropine sulphate, hexamethonium bromide (Sigma), FK 33-824 (Sandoz), D-Met^2-Pro^5-enkephalin, substance P (Peninsula).

Values are given as mean ± s.e.mean.

Results

Response to 20 Hz electrical stimulation

The response of the gut to 20 Hz electrical stimulation while in the presence of atropine (2×10^{-6} M) is illustrated in Figure 1. This contracture could be elicited repeatedly without observing a significant diminution in magnitude provided the period of electrical stimulation was kept short and a 5 min rest period was allowed between responses. Pretreatment with tetrodotoxin (TTX, 1 μg/ml) or desensitization to substance P inhibited this contracture by 75.3 ± 8.3% (mean ± s.e.mean) and 76.3 ± 10.1% respectively ($n = 10$; Figure 1).

Effect of morphine and opioid peptides on responses to 20 Hz stimulation

The effects of (−)-morphine (5×10^{-6} M) on the atropine-resistant contracture in response to 20 Hz stimulation is illustrated in Figure 2. (−)-Morphine (5×10^{-6} M) produced a 24.8 ± 6.5% ($n = 8$) inhibition of the magnitude of the contracture. The threshold for this inhibitory effect was 10^{-6} M and maximum inhibition was produced with a concentration of about 10^{-5} M. An inhibition of greater than 60% was never observed. In contrast (+)-morphine in similar high concentrations (10^{-5} M had no effect on the 20 Hz contracture (Table 1)). Table 1 also illustrates that the opioid peptides D-Ala^2-D-Leu^5 enkephalin (5×10^{-6} M), FK 33-824 (9×10^{-7} M) and D-Met^2-Pro^5-enkephalin (3×10^{-7} M) were also able to inhibit the magnitude of the atropine-resistant 20 Hz contracture. The inhibition produced by either

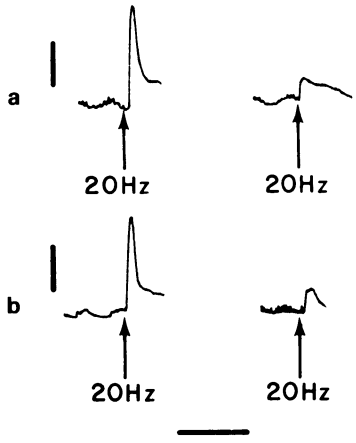

Figure 1 Effects of tetrodotoxin (TTX) and substance P desensitization on the noncholinergic contracture evoked by 20 Hz stimulation. An intact segment of ileum was removed and mounted as described in methods. Following atropine pretreatment and while still in its presence (2 μM) the preparation was stimulated at a frequency of 20 Hz until a maximum response was obtained. Responses were elicited once every 5 min until the magnitude of 3 successive contractures were within 5% of each other. The preparation was then pretreated for 20 min with TTX (1 μg/ml) (a) or desensitized to substance P as described in methods (b) after which it was again stimulated (20 Hz). One of ten similar experiments. Vertical calibration is 0.5 g. Horizontal calibration is 30 s.

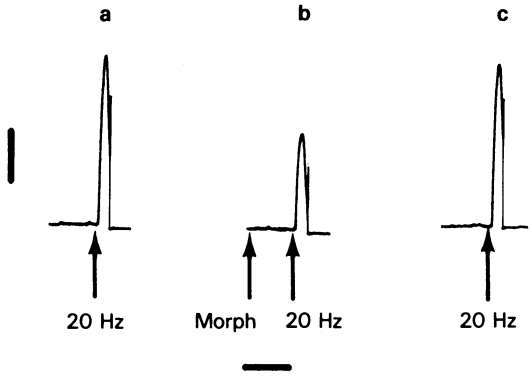

Figure 2 Effects of (−)-morphine (Morph) on the magnitude of the contracture elicited by 20 Hz stimulation. (a) Control responses to 20 Hz stimulation were established as described in the legend to Figure 1. The preparation was then pretreated for 2 min with morphine (5×10^{-6} M) after which it was again stimulated at 20 Hz (b). Following 3 washes and a 5 min rest period, a control response was again elicited (c). One of 15 experiments. Vertical calibration is 0.5 g. Horizontal calibration is 2 min. Chart speed was increased 5 fold during 20 Hz stimulation.

morphine or the opioid peptides was significantly reduced by pretreatment with naloxone (10^{-6} M).

Effect of morphine on contractile responses induced by exogenous substance P

The magnitude of the atropine-resistant contractile response to exogenous substance P (4×10^{-11} M–4×10^{-10} M) was determined before and after a 2 min incubation with morphine (5×10^{-6} M). Morphine was without any significant effect on this response ($P>0.5$ for all concentrations tested, $n=4$ for each concentration). This concentration of morphine was chosen since it was the one against which the potencies of the peptides were compared and it was the concentration that was used in most of the experiments. The effects of morphine on the rate of decay of the substance P-induced increase in tension was also determined to explore further whether opiates could alter the response of the ileum to exogenous substance P. Three min after the administration of substance P (10^{-10} M) 19.8±1.1% of the maximum increase in tension remained ($n=4$, 15 determinations) as compared with 22.0±2.8% when morphine was given 1 min after challenge with substance P. Thus morphine did not significantly alter the rate of decay of the substance P-induced contracture ($n=4$, 10 determinations; $P>0.4$).

Effects of hexamethonium pretreatment

Experiments were conducted to determine whether the inhibition produced by morphine on the atropine-resistant 20 Hz contracture involved a nicotinic cholinergic mechanism. Pretreatment with the nicotinic receptor blocker, hexamethonium (10^{-5} M) reduced the magnitude of the response to 20 Hz stimulation by 36.8±2.5% (Figure 3). No additional inhibition of response to 20 Hz stimulation was observed when higher concentrations of hexamethonium were used (10^{-4} M; $P>0.5$, $n=4$). This pretreatment did not significantly alter responses to exogenous substance P (4×10^{-11} M; $P>0.5$, $n=4$). However, the inhibitory response to morphine, while still in the presence of hexamethonium (10^{-5}–10^{-4} M), was not significantly affected, i.e., 24.8±6.5% before compared with 21.2±1.4% after preincubation with hexamethonium (Figure 3; $n=5$, for each concentration, $p>0.4$). Similarly, hexamethonium pretreatment (10^{-5}–10^{-4} M) did not affect the magnitude of the inhibitory responses to any of the opioid peptides tested ($P>0.5$, $n=3$ for each peptide).

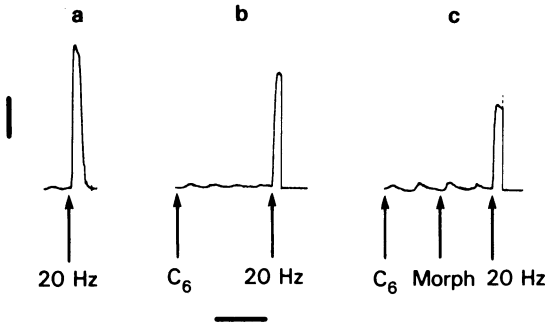

Figure 3 Effects of pretreatment with hexamethonium (C_6) on the magnitude of the atropine-resistant 20 Hz contracture and on the inhibitory effects of (−)-morphine (Morph). (a) Atropine-resistant control responses to 20 Hz stimulation were established as described in Figure 1. The preparation was then pretreated for a minimum of 4 min with hexamethonium (10^{-5} M) after which contractures to 20 Hz stimulation were elicited at 5 min intervals until the magnitude of 3 successive contractures were within 5% of each other (b). While in the presence of hexamethonium (10^{-5} M) the preparation was pretreated for 2 min with morphine (5×10^{-6} M) after which the magnitude of the 20 Hz contracture was determined (c). Atropine was present throughout the entire experiment. Vertical calibration is 0.5 g. Horizontal calibration is 2 min. Chart speed was increased 5 fold during electrical stimulation. One of 5 experiments.

Effect of naloxone on the response to 20 Hz stimulation

The effects of naloxone pretreatment on the magnitude of the atropine-resistant 20 Hz contracture were studied in order to determine whether endogenous opioids could also modulate noncholinergic excitatory responses to electrical stimulation. Figure 4 illustrates that naloxone (10^{-6} M) enhanced the magnitude of response to 20 Hz stimulation. In a total of 18 experiments (43 determinations) naloxone produced a 15.4±2.7% increase in response. The magnitude of this response to naloxone was found to vary from no effect in 3 experiments to increases of greater than 50% in 2 experiments; in one experiment an increase of 93% was observed. Pretreatment with naloxone (10^{-6} M) did not affect responses to exogenous substance P (4×10^{-11}–4×10^{-10} M, $P>0.5$ for all concentrations, $n=3$ for each concentration). The magnitude of the facilitation caused by naloxone was not affected when determined 5 min after starting exposure to hexamethonium (10^{-5}–10^{-4} M; $P>0.5$, $n=3$). In addition it was found that naloxone did not enhance the amplitude of the atropine-resistant contracture after desensitization of the preparation to substance P.

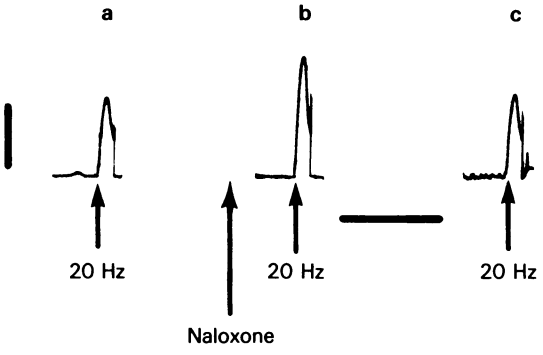

Figure 4 Effects of naloxone pretreatment (10^{-6} M) on the magnitude of response of naïve ileum to 20 Hz stimulation. Control responses to 20 Hz stimulation were obtained as described above (a). The preparation was then pretreated for 2 min with naloxone (10^{-6} M) after which it was again stimulated at 20 Hz (b). The ileum was washed 3 times and allowed to rest for 5 min after which a control response was again elicited (c). Vertical calibration is 1.0 g. Horizontal calibration is 2 min. Chart speed was increased 5 fold during the 20 Hz stimulation. One of 18 similar experiments.

Discussion

Transmural field stimulation (10–20 Hz) of an atropine-pretreated intact segment of guinea-pig ileum, produces a contracture that can be elicited repeatedly without significant variation in magnitude. An appreciable portion of this response is nerve mediated since pretreatment with TTX (1 μg/ml) abolishes about 75% of the response. TTX blocks action potentials in nerve but not smooth muscle and so is an effective tool for distinguishing nerve mediated effects of drugs from direct effects on smooth muscle (Gershon, 1967).

Because of the lack of specific substance P antagonists, it is necessary to use desensitization to substance P as an alternative means by which to demonstrate that a pharmacological response is mediated via the release of this peptide. Substance P desensitization has been shown to be effective in reducing responses of the isolated ileum of the guinea-pig to exogenous substance P, in a highly specific fashion (Costa, Franco & Furness, 1978; Franco, Costa & Furness, 1979; Gintzler, 1980). The ability of this pretreatment to reduce substantially the nerve-mediated component of responses to 20 Hz stimulation (Figure 1) coupled with the known sensitivity of gut smooth muscle to the spasmogenic actions of substance P (Rosell, Bjorkroth, Chang, Yamaguchi, Wan, Rackur, Fisher & Folkers, 1977) strongly indicate that this peptide is the excitatory transmitter that mediates a substantial portion, if not all, of the 20 Hz contracture. These results are in complete agreement with the observations of Franco *et al.* (1979).

Morphine (10^{-6} M–10^{-5} M) produced a concentration-dependent inhibition of the magnitude of the 20 Hz contracture. Since these concentrations of morphine did not significantly decrease excitatory responses to exogenous substance P, it appears likely that this inhibitory effect of morphine is due to the drug's ability to modulate the electrically evoked release of this peptide. However, direct quantitative measurement of the release of this peptide will be necessary in order to demonstrate this effect unequivocally. The ability of opiates to inhibit release of substance P is not without precedent. Jessel & Iversen (1977) have reported a similar effect of morphine on the release of substance P in the spinal trigeminal nucleus.

The inhibition produced by morphine on the magnitude of the noncholinergic contracture has several

Table 1 The effects of morphine and opioid peptides on the atropine-resistant 20 Hz contracture (determined as described in the legend to Figure 1)

Drug tested†	*% inhibition*††	*% inhibition after pretreatment with naloxone* (10^{-6} M)
(−)-Morphine (5×10^{-6} M)	24.8 ± 6.5 $n = 15$ (30)	*4.0 ± 1.6 $n = 7$ (14)
(+)-Morphine (10^{-5} M)	0 $n = 5$ (10)	
D-Ala^2,D-Leu^5-enkephalin (5×10^{-6} M)	24.1 ± 3.5 $n = 3$ (6)	*0 $n = 3$ (6)
FK 33-824 (9×10^{-7} M)	25.6 ± 2.6 $n = 3$ (6)	*13.5 ± 0.5 $n = 3$ (6)
D-Met^2-Pro^5-enkephalin (3×10^{-7} M)	24.1 ± 3.5 $n = 3$ (6)	*9.8 ± 3.2 $n = 3$ (6)

†Each drug was added to the organ bath 2 min before electrical stimulation.
††Results are given as the mean% inhibition of contracture height ± s.e.mean%. The number of individual determinations for each drug is indicated in parentheses.
*$P < 0.01$

of the characteristics of previously described opiate receptor-mediated effects. The inhibition appears to be stereospecific in that (+)-morphine, in twice the concentration used for (−)-morphine, is devoid of any inhibitory activity. Furthermore the inhibition produced by (−)-morphine and the opioid peptides is significantly antagonized by naloxone (Table 1). A naloxone:morphine concentration ratio of 1:5 produced an 84% reversal of the morphine-induced inhibition. This is very similar to the degree of reversal (73%) that is produced when the same relative concentration of naloxone is used as an antagonist of morphine in this preparation stimulated at 0.1 Hz.

Several experimental approaches have suggested the existence of a multiplicity of opiate receptor types. Experiments in chronic spinal dog preparations (Martin, Eades, Thompson, Huppler & Gilbert, 1976) indicated the presence of three subclasses of opioid receptor designated μ, κ and δ. The existence of a heterogeneous population of opiate receptors is also supported by experiments in which the potencies of various ligands were compared in parallel assay in different systems (Lord, Waterfield, Hughes & Kosterlitz, 1977; Leslie, Chavkin & Cox, 1980). The experiments presented in this manuscript do not allow for any definitive conclusions concerning the nature of the opiate receptor that appears to be involved in modulating release of enteric substance P. Such conclusions would require a detailed analysis of the relative potency of naloxone in antagonizing the inhibition produced by the various ligands as well as the determination of the relative potency of a greater number of opioid ligands to known selectivity for the various receptor subtypes.

Pretreatment with hexamethonium (10^{-5}–10^{-4} M), a nicotinic receptor blocker, was found to reduce by 36.8% the magnitude of response to 20 Hz stimulation. This suggests that a portion of the electrically evoked release of substance P is secondary to the release of ACh and the activation of nicotinic ganglionic receptors. This is consistent with the observation that the contracture evoked by 1,1-dimethyl-4-phenylpiperazinium (DMPP), a nicotinic ganglionic agonist, can be partially blocked (25%) by substance P desensitization (Franco *et al.*, 1979; Gintzler, unpublished observations). However, the failure of hexamethonium pretreatment (in concentrations in excess of that needed to inhibit the peristaltic reflex, 10^{-4} M) to reduce the inhibition that morphine produces on the magnitude of the 20 Hz contracture (Figure 3) indicates that this inhibition does not involve a nicotinic cholinergic mechanism.

Figure 1 indicates that about 25% of the contracture elicited by 20 Hz stimulation is resistant to TTX (1 μg/ml). Thus, the 20 Hz contracture is a mixed neurogenic and myogenic response that could represent the release of transmitter by sodium-dependent nerve action potentials, the direct release of transmitter by the applied voltage field and the direct electrical stimulation of smooth muscle. This makes it somewhat difficult to deduce the precise mechanism by which the opioids and opioid peptides reduce the magnitude of the 20 Hz contracture. It appears unlikely that morphine is affecting the component of the noncholinergic contracture that is due to direct stimulation of smooth muscle cells. Morphine does not affect the direct stimulation of gut smooth muscle by exogenous ACh (Paton, 1957) or substance P (this paper). Furthermore, morphine was able to produce as much as a 60% inhibition of the 20 Hz contracture whereas the TTX-resistant component never exceeded 25% of the control response. Thus, even if morphine did affect the myogenic component of contracture, an interaction with a noncholinergic excitatory neurone(s) must still be considered. However, it is not clear whether morphine is inhibiting the release of transmitter caused by sodium-dependent nerve action potentials or whether it is reducing the release of transmitter caused by the direct application of the voltage field.

The concentration of morphine required to inhibit noncholinergic excitatory responses (10^{-6} M–10^{-5} M) is significantly higher than the concentration needed to inhibit the electrically induced release (0.1 Hz) of ACh ($IC_{50} = 10^{-7}$ M). However, it should be noted that high frequency-induced release of ACh is much less sensitive to inhibition by morphine than is low frequency-induced release (Paton, 1957). The noncholinergic excitatory response described here is elicited by high frequency stimulation (10–20 Hz). It is also possible that the inhibitory effects of opiates on the 20 Hz contracture is mediated via an opiate receptor subtype other than the μ receptor. In this case morphine would not be expected to have a potency comparable to its effect on the release of ACh, an action that is mediated via a μ receptor (Lord *et al.*, 1977). Lastly, since electrically evoked release of enteric substance P is under endogenous endorphinergic inhibition (Figure 4) it is difficult to construct an accurate morphine dose-response relationship for its inhibitory effect on the noncholinergic contracture.

Naloxone (10^{-6} M) was found to enhance atropine-resistant responses to 20 Hz stimulation without affecting the magnitude of response to exogenous substance P. Moreover this stimulatory effect of naloxone was not affected by hexamethonium pretreatment (10^{-5}–10^{-4} M). This strongly suggests that naloxone is acting directly to enhance release of substance P and that endogenous opioids may act *in situ* to regulate the release of this enteric peptide. Thus endorphin-containing neurones in the enteric

nervous system appear to play an important role in modulating, directly or indirectly, the activity of at least two different types of excitatory neurone, cholinergic and peptidergic (substance P).

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