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Contractor responses of the isolated colon of the mouse to morphine and some opioid peptides

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1 Morphine ($1 \times 10^{-8} - 1 \times 10^{-4}$M), fentanyl ($1 \times 10^{-9} - 1 \times 10^{-5}$M) and alfentanyl ($1 \times 10^{-10} - 1 \times 10^{-5}$M) as well as methionine enkephalin [Met^5]enkephalin ($1 \times 10^{-11} - 1 \times 10^{-8}$M), [D-Ala^2, Met^5]enkephalin ($1 \times 10^{-12} - 1 \times 10^{-8}$M) and dynorphin A(1 – 13) ($1 \times 10^{-9} - 1 \times 10^{-6}$M) caused a contractor response of the longitudinal musculature of the terminal colon of the mouse.

2 These effects were competitively antagonized by naloxone. The pA_2 values obtained for naloxone antagonism of morphine and opioid peptides and the high sensitivity of the preparation to enkephalins suggest the presence of δ-opiate receptors in this preparation but μ- and κ-receptors may also be present.

3 Opiate-induced contractions in the mouse colon were abolished by tetrodotoxin and after incubation with indomethacin.

4 It is concluded that the excitatory actions of the opiates in the mouse colon are mediated via opiate receptors located on nerves which do not release acetylcholine, noradrenaline or 5-hydroxytryptamine. The opiates may produce their action by removing an inhibitory neural influence (the nature of which remains to be elucidated) allowing a prostaglandin-mediated effect to predominate, thereby increasing muscle tone.

Introduction

The presence of opioid peptides has been demonstrated in several parts of the gastrointestinal tract of different species, and they may have a neurotransmitter or neuromodulator role in the enteric nervous system (Hughes *et al.*, 1977).

In the guinea-pig isolated ileum, morphine and the opioid peptides inhibit the release of acetylcholine via the activation of prejunctional opiate receptors (Paton, 1957; Waterfield *et al.*, 1977). In contrast, morphine may increase the contractility of dog and rat intestine by releasing 5-hydroxytryptamine (Burks, 1973; 1976). Excitatory effects of morphine, enkephalins and endorphins have been more recently described in the isolated colon of the rat (Gillan & Pollock, 1980; Nykamp & Van Ree, 1980; Huidobro-Toro & Way, 1981). In the present study, we have investigated the effects of morphine, some narcotic drugs (fentanyl, alfentanyl) and some opioid peptides methionine-enkephalin ([Met^5]enkephalin), [D-Ala^2, Met^5]enkephalin, dynorphin A (1–13) in the isolated terminal colon of the mouse.

Methods

Swiss Webster mice (30–40 g) were stunned and killed by exsanguination; the terminal colon was dissected out and placed under an initial load of 1 g in an organ bath containing Krebs solution of the following composition (mM): NaCl 118.1, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 5. The bath (25 ml) was maintained at 36 ± 1°C and bubbled continuously with a mixture of 95% O_2 and 5% CO_2. The isometric contractions of the longitudinal muscle were measured with a Grass force-displacement transducer. Preparations were allowed to equilibrate in Krebs solution for at least 60 min before drugs were added. Acetylcholine (ACh) (1×10^{-6}M) was added at the beginning of each experiment to test the reactivity of the preparation. The morphine-like drugs were left in contact with the tissue for periods of 2–3 min at intervals of 15 min. Antagonists were incubated for at least 10 min before agonists were tested ($n = 4-6$).

Dose-response curves were constructed for each

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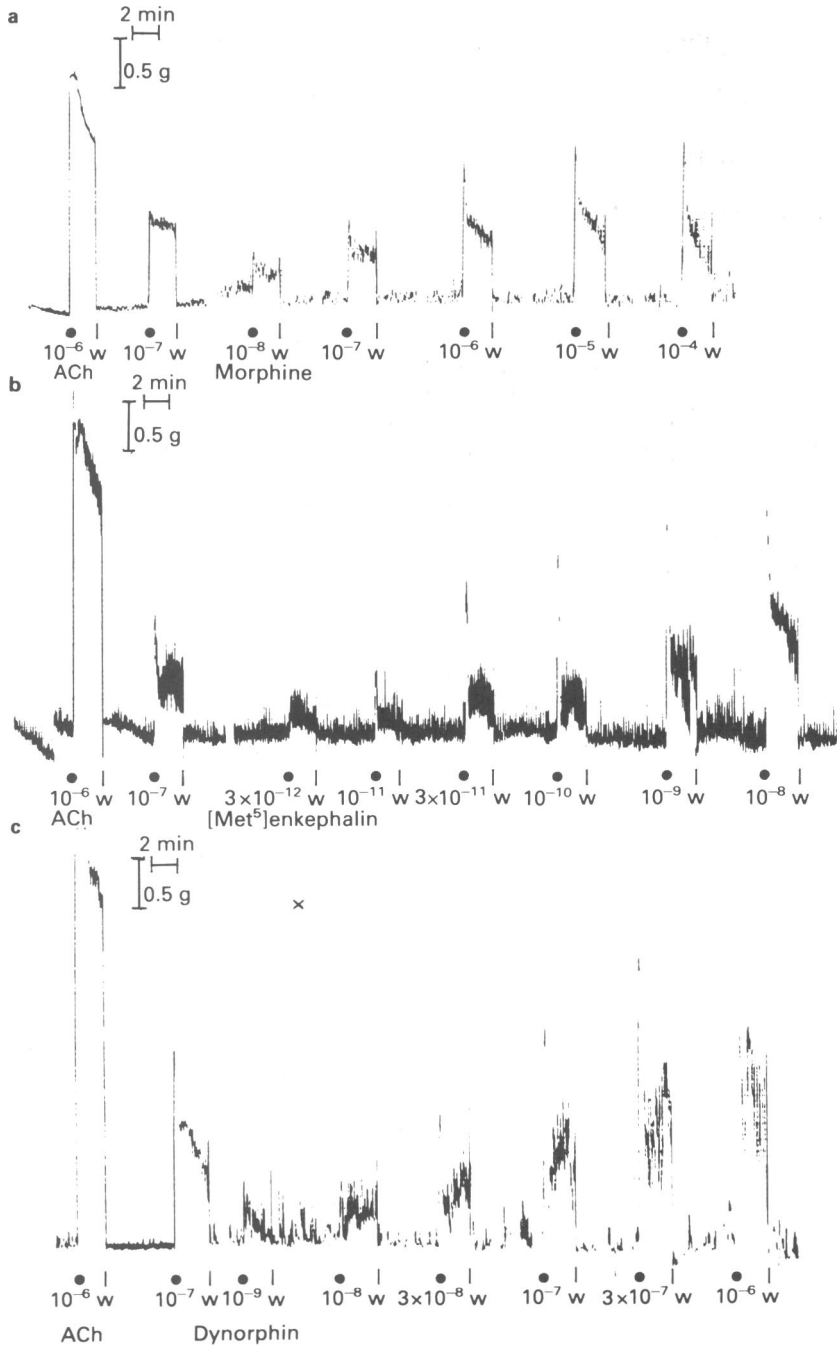

Figure 1 Contractor responses to morphine of the isolated colon of the mouse (a), methionine enkephalin [Met^5]enkephalin (b) and dynorphin A (1–13) (c). Drugs were given at 15 min intervals. Acetylcholine (ACh) was added to test the reactivity of the preparation. W = washout.

Table 1 Sensitivity of the mouse colon to morphine-like drugs

Agonists	*Maximum contractor responses* (g)	EC_{50} (M)	pD_2 ($-\log EC_{50}$)
Morphine	(7) 1.80 ± 0.10	$5.1\ 10^{-7}$ ± 2.0	6.2
Fentanyl	(8) 2.12 ± 0.15 NS	$6.9\ 10^{-8}$ ± 4.2	7.1
Alfentanyl	(8) 2.0 ± 0.17 NS	$2.1\ 10^{-8}$ ± 0.5	7.6
[Met^5]enkephalin	(7) 2.15 ± 0.20 NS	$3.7\ 10^{-10}$ ± 1.2	9.4
[D-Ala^2, Met^5] enkephalin	(8) 2.20 ± 0.17 NS	$2.5\ 10^{-11}$ ± 0.7	10.6
Dynorphin A (1–13)	(10) 1.85 ± 0.12 NS	$2.7\ 10^{-8}$ ± 0.5	7.5

Number in parentheses indicate number of concentration-effect curves determined in different preparations. Values are given as means ± s.e.mean NS = not significantly different from values for morphine (Student's t test).

opioid ($n = 7–10$). The mean maximal contractor response (in g) (± s.e.mean) and the mean EC_{50} (in M) (± s.e.mean) were determined from these experiments. Results were analysed by use of Student's t test.

Dose-response curves to morphine, [met^5] enkephalin and dynorphin A (1–13) were also constructed in the presence of 2 or 3 concentrations of naloxone ($1 \times 10^{-8} - 3 \times 10^{-5}$M) ($n = 4$ for each concentration) and the pA_2 values of this antagonist were calculated by a Schild plot.

Drugs

The following drugs were used: acetylcholine hydrochloride (Astra), atropine sulphate (Fluka), indomethacin (Merck Sharp & Dohme), mepyramine maleate (Rhone-Poulenc), methysergide hydrogenmaleate (Sandoz), naloxone hydrochloride (Endo), phentolamine methanesulphonate (Ciba), (±)-propranolol hydrochloride (ICI), tetrodotoxin crystalline 3x (Calbiochem), morphine hydrochloride (Bios Coutelier), fentanyl (Janssen), alfentanyl (Janssen), methionine-enkephalin ([Met^5]enkephalin) (Peninsula), [D-Ala^2, Met^5]enkephalin (Peninsula), dynorphin A (1–13) (Peninsula). Indomethacin (31 mg) was dissolved in ethanol (4 ml) and diluted with distilled water to give 0.31 mg ml^{-1}; 0.05 ml of this solution was added to the organ bath. Concentrations of drugs (M) refer to molar concentration of their salts in the bath.

Results

Contractor responses to morphine, fentanyl and alfentanyl

Morphine ($1 \times 10^{-8} - 1 \times 10^{-4}$M) produced dose-dependent contractions of the longitudinal muscular layer of the mouse colon (Figure 1a). The responses were rapid in onset, an initial peak being followed by a more sustained response. They were reproducible at 15 min intervals. The mean EC_{50} values and pD_2 ($-\log EC_{50}$) and the mean maximal tensions (in g) developed were calculated from the concentration-effect curves and are shown in Table 1.

As shown in Figure 1 the maximal response evoked by morphine (1×10^{-4}M) was lower (50.6% ± 7.3, $n = 5$, mean ± s.e.mean) than that evoked by ACh (1×10^{-6}M), in the same experiments. Fentanyl ($1 \times 10^{-9} - 1 \times 10^{-5}$M) and alfentanyl ($1 \times 10^{-10} - 1 \times 10^{-5}$M) were as active as morphine in producing a contraction of the mouse colon; furthermore, the concentration-effect curves of the three drugs were parallel. The EC_{50}s of fentanyl and alfentanyl were about ten times lower than that of morphine, the maximum tensions induced by them, though often higher, were not significantly greater (Table 1).

Contractor responses to opioid peptides

[Met^5]enkephalin ($3 \times 10^{-12} - 1 \times 10^{-8}$M) (Figure 1b) and [D-Ala^2, Met^5]enkephalin ($1 \times 10^{-12} - 1 \times 10^{-8}$M) (not shown) respectively contracted the mouse colon in the same way as morphine and the spontaneous phasic activity of the preparation was generally enhanced between successive additions of enkephalins to the bath. The EC_{50}, pD_2 and maximum tensions developed were obtained from concentration-effect curves and shown in Table 1.

The maximum responses induced by enkephalins were not significantly greater than those induced by morphine, despite being about 20% higher. Dynorphin A (1–13) (Figure 1c) also contracted the preparation in a dose-dependent manner but at higher concentrations. Its EC_{50} and pD_2 values and the maximum tension induced by it are shown in Table 1.

Inhibitory effects of naloxone

Naloxone in concentrations up to 3×10^{-5}M did not induce a contractor effect of the mouse colon but was a

Table 2 Inhibitory effects of naloxone in the mouse colon

Agonist	*Naloxone pA_2 values*	*Slope*
Morphine	9.12 ± 0.18 (8)	0.89
[Met^5]enkephalin	7.60 ± 0.08 (12)	1.16
Dynorphin A (1–13)	5.88 ± 0.12 (8)	1.11

Mean values are given ± s.e.mean.
Numbers in parentheses indicate number of determinations.

potent antagonist of the opioid-induced responses.

Naloxone displaced to the right in a parallel and dose-related fashion the concentration-effect curves of morphine, [Met^5]enkephalin and dynorphin A (1–13). The pA_2 values obtained for these drugs are shown in Table 2 and were considerably higher for morphine than for [Met^5]enkephalin and dynorphin A (1–13).

Effect of non-opiate receptor antagonists

The contractile responses induced by morphine (3×10^{-7}M), [Met^5]enkephalin (3×10^{-9}M) and dynorphin A 1–13 (10^{-7}M) were reproducible at 15 min intervals and not modified by atropine (3×10^{-7}M), mepyramine (2.5×10^{-7}M), methysergide (5×10^{-7}M), propranolol (1×10^{-6}M) or phentolamine (3×10^{-6}M). They were abolished by low concentrations of tetrodotoxin (1×10^{-7}M) (Figure 2) while the ACh-induced contractions were not modified. The responses to the morphine-like drugs were restored 30 min after washing out tetrodotoxin

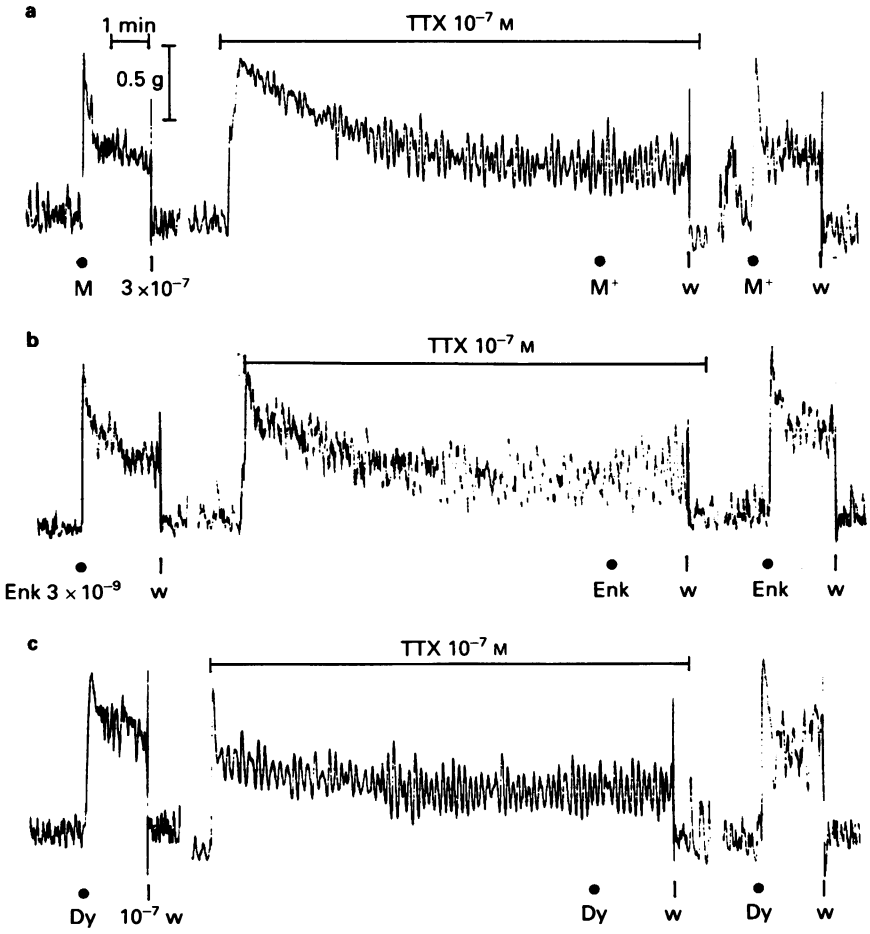

Figure 2 Inhibitory effects of tetrodotoxin (TTX) 1×10^{-7}M on the contractions induced by morphine (M), [Met^5]enkephalin (Enk) and dynorphin A (1–13) (Dy) on the mouse colon. The responses to the agonists were restored 30 min after washing out tetrodotoxin. W = washout.

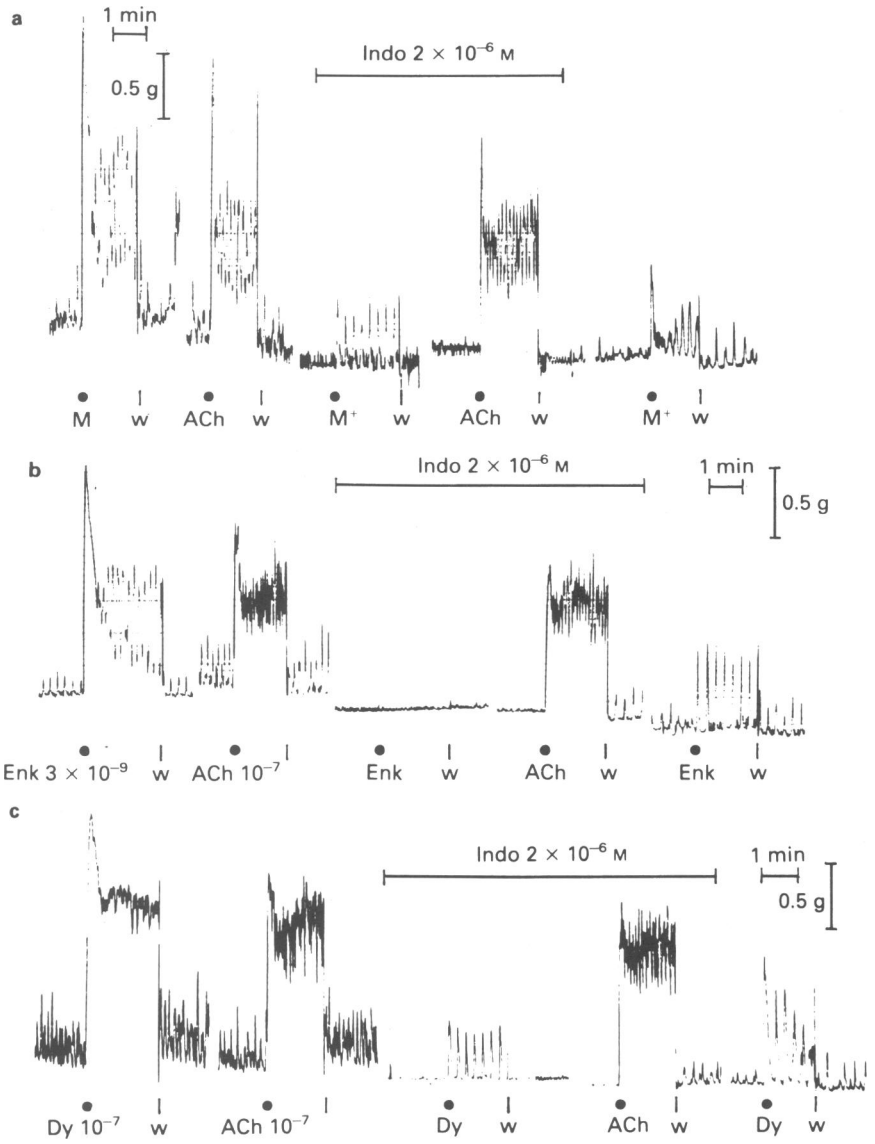

Figure 3 Inhibitory effects of indomethacin (Indo) 2×10^{-6} M on the contractile activity of morphine (M), [Met^5]enkephalin (Enk) and dynorphin A (1–13) (Dy). The acetylcholine (ACh)-induced responses were not modified under the same experimental conditions. The responses to the morphine-like drugs were poorly restored 30 min after washing out indomethacin. W = washout.

(six intermediate washings of the preparation at 5 min intervals). As previously described (Fontaine *et al.*, 1984) tetrodotoxin, itself, caused a sustained contraction of the colon and increased spontaneous activity (Figure 2).

Contractions induced by morphine, [Met^5]enkephalin or dynorphin A (1–13) (Figure 3) were abolished by exposure to indomethacin (2×10^{-6}M) for 20 min.; phasic activity, however, still appeared (Figure 3). The responses to acetylcholine were not modified by indomethacin (Figure 3) and the solvent for indomethacin (40% ethanol in water) had no effect. The inhibitory effects of indomethacin were very slowly reversible: the responses to the morphine-like drugs were poorly restored 30 min after discontinuing exposure to indomethacin (Figure 3).

Discussion

The present results demonstrate the existence of excitatory opiate receptors in the mouse colon. These receptors can be stimulated by low concentrations of morphine or other opiates, e.g. fentanyl and alfentanyl, two potent narcotic agonists (Gardocki & Yelnosky, 1964; Niemegeers & Janssen, 1981), as well as by the opioid peptides [Met^5]enkephalin, [D-Ala^2, Met^5]enkephalin and dynorphin A (1–13). The contractor responses induced by these agonists were competitively antagonized by naloxone, a pure opiate antagonist.

[D-Ala^2,Met^5]enkephalin and [Met^5]enkephalin were the most potent agonists. Although care should be taken in the comparison of potencies of opioid peptides in relation to differing sensitivity to enzymatic degradation, the increased potency of [D-Ala^2, Met^5]enkephalin relative to [Met^5]enkephalin is likely to be due to increased metabolic stability of the [D-Ala^2]-substituted analogue (Pert *et al.*, 1976; McKnight *et al.*, 1983). Fentanyl, alfentanyl and dynorphin A (1–13) were essentially equipotent in causing contraction of the mouse colon and, as in the guinea-pig ileum, were more potent than morphine (Fennessy *et al.*, 1969, Yoshimura *et al.*, 1982). The pA_2 values obtained in the mouse colon for naloxone antagonism of morphine, [Met^5]enkephalin and dynorphin A (1–13) were not equivalent. Indeed, the antagonism of the opioid peptides required much higher concentrations of naloxone than that of morphine. This contrasts with the results obtained in the guinea-pig ileum where [Met^5]enkephalin and morphine were equipotent in depressing electrically-evoked contractions and naloxone antagonized the enkephalins as readily as morphine (Waterfield *et al.*, 1977). The different potencies of opioids in various peripheral tissues have been explained by the concept of multiple opiate receptors (Lord *et al.*, 1977: Wüster *et al.*, 1981). Morphine and the narcotic agonist, fentanyl or analogues exhibit a preference for μ-receptors, [Met^5]enkephalin and [D-Ala^2, Met^5]enkephalin for δ-receptors. However, in the guinea-pig ileum, enkephalins are thought to act via μ-opiate receptors (Lord *et al.*, 1977). On the other hand dynorphin A (1–13) has been demonstrated to act as a κ-receptor agonist in *in vitro* isolated preparations such as guinea-pig and rabbit ilea, mouse and rabbit vasa deferentia (Huidobro-Toro *et al.*, 1981; Oka *et al.*, 1982).

The high sensitivity of the mouse colon to enkephalins and the naloxone pA_2 value for this peptide suggest the presence of δ-opiate receptors in this preparation, as previously shown in other isolated intestinal preparations, including the rat colon (Boura & Olley, 1981) the rat, mouse and rabbit ilea (Oka, 1981) and the rat rectum (Shaw, 1979). Although the observations with morphine and dynorphin A (1–13) suggest the possible involvement of μ- and κ-receptors in the mouse colon, more conclusive evidence would require the use of more μ-selective or κ-selective agonists and antagonists. The pharmacological analysis that we have made to elucidate the mechanism of the excitatory effect of the opiates in the colonic musculature of the mouse indicates that they act on the intramural nerve plexus of the colon and that prostaglandins are probably involved in the observed contractor response.

Indeed, the opiate-induced contractions were abolished by tetrodotoxin, which blocks conducted neuronal activity (Gershon, 1967) and by indomethacin which inhibits prostaglandin synthesis (Vane, 1971).

A neuronal site of action of morphine and opioid peptides had been previously described in the rat colon *in vitro* (Huidobro-Toro & Way, 1981; Yau, 1981). Burks (1973; 1976) demonstrated that the narcotic analgesics release 5-hydroxytryptamine from the small intestine preparation of the dog or rat, thus explaining the excitatory action of morphine in the small bowel of these species. More recent data demonstrated that morphine and opioid peptides can influence gastrointestinal motility by activation of opiate receptors located within the central nervous system (Galligan & Burks, 1982; Porreca *et al.*, 1984). In the rat colon, the results concerning a role of 5-hydroxytryptaminergic neurones in the contractile effects of opiates are controversial (Gillan & Pollock, 1980; Nÿkamp & Van Ree, 1980; Huidobro-Toro & Way, 1980; Yau, 1981).

In the mouse colon, morphine effects may not be mediated by the local release of 5-hydroxytryptamine, acetylcholine, histamine or catecholamines since they are not modified by methysergide, atropine, mepyramine, phentolamine or propranolol. We have previously shown (Fontaine *et al.*, 1984) that in this preparation, muscle tone and contractility are regulated by two opposing mechanisms, namely (1) a neurogenic cholinergic activity and a local prostaglandin synthesis leading to an increase in muscle tone and (2) a neurogenic non-adrenergic, non-cholinergic (NANC) inhibitory control (the nature of which remains to be elucidated). From the present results, presynaptic inhibitory opiate receptors should be located on NANC inhibitory nerves which normally suppress myogenic activity.

A similar hypothesis has been proposed previously for the rat colon (Gillan & Pollock, 1980) and the rabbit colon (Blanquet *et al.*, 1982). Moreover, morphine has been shown to depress the non-adrenergic inhibitory responses of guinea-pig taenia coli to transmural stimulation (Shino & Ishu, 1978).

In the mouse colon, morphine and opioid peptides, by blocking the NANC inhibitory pathway, would allow a prostaglandin-induced effect to predominate. This would lead to a contraction of the preparation.

The role of prostaglandins in the contractility of the mouse colon has been described previously (Fontaine *et al.*, 1984; Fontaine & Lebrun 1985). Since the opioid-induced contractions are inhibited in the same way as sympathomimetically-induced contractions (i.e. by tetrodotoxin and indomethacin), it is tempting to assume that the opiate receptors and adrenoceptors are located on the same neuronal pathway.

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References

BLANQUET, F., BOUVIER, M. & GONELLA, J. (1982). Effects of enkephalins and morphine on spontaneous electrical activity and on junction potentials elicited by parasympathetic nerve stimulation in cat and rabbit colon. *Br. J. Pharmac.*, **77**, 419–429.

BOURA, A.L.A. & OLLEY, J.E. (1981). Characterization of the excitatory opiate receptors in the rat large intestine. *Br. J. Pharmac.*, **74**, 246–247P.

BURKS, T.F. (1973). Mediation by 5-hydroxytryptamine of morphine stimulant actions in dog intestine. *J. Pharmac. exp. Ther.*, **185**, 530–539.

BURKS, T.F. (1976). Acute effects of morphine in rat intestinal activity. *Eur. J. Pharmac.*, **40**, 279–283.

FENNESSY, M.R., HEIMANS, R.L.H. & RAND, M.J. (1969). Comparison of effect of morphine-like analgesics on transmural stimulated guinea-pig ileum. *Br. J. Pharmac.*, 37, **436–449.**

FONTAINE, J., GRIEVEGNEE, A. & REUSE, J. (1984). Adrenoceptors and regulation of intestinal tone in the isolated colon of the mouse. *Br. J. Pharmac.*, **81**, 231–243.

FONTAINE, J. & LEBRUN, Ph. (1985). Effects of neurotensin on the isolated mouse distal colon. *Eur. J. Pharmac.*, **107**, 141–147.

GALLIGAN, J.J. & BURKS, T.F. (1982). Opioid peptides inhibit intestinal transit in the rat by a central mechanism. *Eur. J. Pharmac.*, **85**, 61–68.

GARDOCKI, J.F. & YELNOSKI, J. (1964). A study of some of the pharmacologic actions of fentanyl citrate. *Tox. appl. Pharmac.*, **6**, 48–62.

GERSHON, M.D. (1967). Effects of tetrodotoxin on innervated smooth muscle preparations. *Br. J. Pharmac.*, **29**, 259–279.

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

HUGHES, J., KOSTERLITZ, H.W. & SMITH, T.W. (1977). The distribution of methionine-enkephalin and leucine-enkephalin in the brain and peripheral tissue. *Br. J. Pharmac.*, **61**, 639–647.

HUIDOBRO-TORO, J.P. & WAY, E.L. (1981). Contractile effect of morphine and related opioid alkaloids, β-endorphin and methionine enkephalin on the isolated colon from Long Ewans rats. *Br. J. Pharmac.*, **74**, 681–694.

HUIDOBRO-TORO, J.P., YOSHIMURA, K., LEE, N.M., LOH, H. & WAY, E.L. (1981). Dynorphin interaction at the κ-opiate site. *Eur. J. Pharmac.*, **72**, 265–266.

LORD, J.A.H., WATERFIELD, A.A., HUGHES, J. & KOSTERLITZ, H.W. (1977). Endogenous opioid peptides: multiple agonists and receptors. *Nature*, **267**, 495–499.

McKNIGHT, A.T., CORBETT, A.D. & KOSTERLITZ, H.W. (1983). Increase in potencies of opioid peptides after peptidase inhibition. *Eur. J. Pharmac.*, **86**, 393–402.

NIEMEGEERS, C.J.E. & JANSSEN, P.A.J. (1981). Alfentanyl (R39209). A particularly short-acting intravenous narcotic analgesic in rats. *Drug Dev. Res.*, **1**, 83–88.

NYKAMP, F.P. & VAN REE, J.M. (1980). Effects of endorphins on different parts of the gastrointestinal tract of rat and guiniea-pig *in vitro*. *Br. J. Pharmac.*, **68**, 599–606.

OKA, T. (1981). Enkephalin (opiate) receptors in the intestine. *Tips Trends Pharmac. Sci.*, **2**, 328–330.

OKA, T., NEGISHI, K., SUDA, M., SAWA, A., FUJINO, M. & WAKIMASU, M. (1982). Evidence that dynorphin (1–13) acts as an agonist on opioid κ receptors. *Eur. J. Pharmac.*, 77, **137–141.**

PATON, W.D.M. (1957). The action of morphine and related substances on contraction and on acetylcholine output of coaxially stimulated guinea-pig ileum. *Br. J. Phrmac. Chemother.*, **12**, 119–127.

PERT, C.B., BOWIE, D.L., FONG, B.T.W. & CHANG, J.K. (1976). Synthetic analogues of met-enkephalin which resist enzymatic destruction. In *Opiates and Endogenous Opioid Peptides*. ed. Kosterlitz, H.W., pp. 79–86, Amsterdam, N. Holland Publ. C°.

PORRECA, F., MOSBERG, H., HURST, R., HRUBY, V. & BURKS, T.F. (1984). Roles of μ, δ and κ opioid receptors in spinal and supraspinal mediation of gastrointestinal transit effects and hot-plate analgesia in the mouse. *J. Pharmac. exp. Ther.*, **230**, 341–347.

SHAW, J.S. (1979). Characterization of opiate receptors in the isolated rat rectum. *Br. J. Pharmac.*, **67**, 428–429P.

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

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action of aspirin like drugs. *Nature, New Biol.*, **231**, 232–235.

WATERFIELD, A.A., SMOCKUM, R., HUGHES, J., KOSTERLITZ, H.W. & HENDERSON, G. (1977). In vitro pharmacology of the opioid peptides enkephalins and endorphins. *Eur. J. Pharmac.*, **43**, 107–116.

WÜSTER, M., SCHULZ, R. & HERTZ, A. (1981). Multiple opiate receptors in peripheral tissue preparations. *Biochem. Pharmac.*, **30**, 1883–1887.

YAU, W. (1981). Sites of action of enkephalin on rat colon in vitro. *Gastroenterology*, **80**, 1321.

YOSHIMURA, K., HUIDOBRO-TORO, J.P. & WAY, E.L. (1982). Potency of three opiate antagonists to reverse the inhibitory activity of dynorphin, enkephalins and opioid-like alkaloids on the guinea-pig ileum. *Eur. J. Pharmac.*, **84**, 17–24.

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