J. Pharm. Pharmacol. 1993, 45: 668–670
Communicated October 13, 1992

Opioid pathways exert a tonic restraint in the guinea-pig isolated colon: changes after chronic sympathetic denervation

FRANCA MARINO, FILOMENA CRETA, FABRIZIO DE PONTI, CRISTINA GIARONI, SERGIO LECCHINI, GIAN MARIO FRIGO, *Department of Internal Medicine and Therapeutics, Section of Pharmacology and Toxicology, University of Pavia, 2nd Faculty of Medicine, Viale Luigi Borri 57, I-21100 Varese, Italy*

Abstract—We have studied the effects of naloxone on acetylcholine and noradrenaline release in the guinea-pig isolated distal colon, and have assessed the effect of naloxone on electrically-induced contractions of the longitudinal muscle and non-adrenergic, non-cholinergic (NANC) relaxations of the circular muscle coat. Naloxone dose-dependently increased resting and electrically-evoked acetylcholine release and electrically-evoked noradrenaline release. Naloxone was more potent in increasing resting acetylcholine release in colonic specimens obtained after chronic sympathetic denervation. Naloxone (1 μM) did not affect electrically-induced contractions of the longitudinal muscle, while it enhanced NANC relaxations of the circular muscle. The effects observed with naloxone in the present experiments suggest that opioid pathways exert a tonic restraint on neurotransmission in the guinea-pig colon. After suppression of the adrenergic inhibitory tone, the functional relevance of opioid pathways seems to be increased.

Several lines of evidence indicate that the colon is kept under tonic neurogenic inhibition (Roman & Gonella 1987). Previous studies carried out in our laboratory have shown that both the adrenergic and the GABA-ergic systems may contribute to maintain such a tonic restraint in the guinea-pig colon (Marcoli et al 1985; Frigo et al 1987).

Morphological, electrophysiological and pharmacological observations provide compelling evidence that, at least in the ileum, opioids serve as neurotransmitters or neuromodulators in the gastrointestinal tract (Hughes et al 1977; Glass et al 1986; Furness & Costa 1987). Only fragmentary information is available on the possible involvement of opioids in maintaining a tonic restraint on the colon.

To elucidate the possible involvement of opioid pathways in modulating neurotransmission at the colonic level, we have investigated the effect of naloxone on acetylcholine and noradrenaline release in the guinea-pig distal colon. The effect of naloxone on acetylcholine release was also studied after chronic sympathetic denervation, since there is circumstantial evidence that, in the absence of the adrenergic inhibitory tone, adaptive changes may occur and other inhibitory systems may take control (Marcoli et al 1985; Marino et al 1992). Finally, since the action of morphine in the longitudinal and circular muscle has been explained in terms of inhibition of the tonic cholinergic excitatory and non-adrenergic, non-cholinergic (NANC) inhibitory innervation, respectively (Tonini et al 1985), we have studied the effects of naloxone on electrically-induced contractions of the longitudinal muscle and on NANC relaxations of the circular muscle.

Materials and methods

Specimens obtained from the distal colon of male guinea-pigs, 300–400 g, were mounted in organ baths perfused with Tyrode solution at 35·5°C and continuously bubbled with 95% O_2–5% CO_2. The composition of the Tyrode solution was as follows (mM): NaCl 136·9, KCl 2·7, $CaCl_2$ 1·8, $MgCl_2$ 1·04, $NaHCO_3$ 11·9, NaH_2PO_4 0·4 and glucose 5·5. Some experiments were carried out on specimens obtained from animals in which the sympathetic supply to the colon had been removed six days before the experiments (Mazzanti et al 1972).

Acetylcholine release. Endogenous acetylcholine release was measured from 2–3 cm specimens suspended isotonically (tension 10 mN) in a 3 mL organ bath containing Tyrode solution with added physostigmine sulphate (15 μM) according to Frigo et al (1984). Samples of the incubation medium were collected every 20 min after 60 min equilibration (the medium was changed every 20 min). When stimulation-evoked release was measured, four separate collection cycles, each including a 10-min stimulation period (biphasic square pulses; 1 Hz; 1 ms; 450 mA), were carried out every 20 min. Acetylcholine in the incubation medium was assayed on the guinea-pig isolated ileum incubated in Tyrode solution with added physostigmine sulphate (7·7 nM) and morphine sulphate (6·6 μM) according to Paton & Vizi (1969). The concentrations of naloxone in the test samples were duplicated in the standard acetylcholine solutions during the assay to rule out any possible effect of naloxone on the acetylcholine assay.

Noradrenaline release. Noradrenaline release was measured from 2–3 cm colonic segments which had previously been deprived of the mucosa by sharp dissection in order to obtain a neuromuscular preparation and minimize contamination by catecholamines released by the mucosa. The specimens were suspended isotonically (tension 10 mN) in a 3-mL organ bath perfused at the rate of 1 mL min^{-1} with Tyrode solution with added ascorbic acid (0·11 mM) to prevent catecholamine oxidation. Transmural stimulation was obtained by co-axial silver wire electrodes delivering four 30-s trains of pulses (4 Hz; 1 ms; 25–60 V) separated by a rest period of 30 s. The sample collection procedure was similar to that described by Gatti et al (1987). The effect of naloxone was tested by adding the drug to the perfusion fluid for 30 min. Noradrenaline concentrations in the incubation medium were measured by HPLC with coulometric detection (Hjemdahl 1984; Gatti et al 1987), which allows determination of noradrenaline without interference by its metabolites. Recovery of known amounts of catecholamines from the organ bath in the absence of tissue was >90%.

Electrical stimulation. Electrical stimulation was applied to 2–3 cm colonic segments mounted longitudinally and connected to an isotonic transducer (tension 10 mN) in a 10 mL organ bath. Rectangular pulses of submaximal strength (4 Hz; 1 ms; 25–50 V for 20 s) were applied through coaxial silver wire electrodes at 20 min intervals. NANC relaxations were obtained in circular muscle strips deprived of the mucosa and suspended isotonically (tension 2·5 mN). The specimens of circular muscle were pretreated with hyoscine sulphate (3 μM) and guanethidine sulphate (5 μM) and stimulated at 20 min intervals with rectangular pulses of submaximal strength (1–4 Hz; 0·1 ms; 25–50 V for 20 s). After obtaining reproducible responses to electrical stimulation, the effect of 1 μM naloxone was tested allowing a contact period of 20 min.

Correspondence: G. M. Frigo, Department of Internal Medicine and Therapeutics, Section of Pharmacology, 2nd Faculty of Medicine, University of Pavia, Viale Luigi Borri 57, I-21100 Varese, Italy.

Drugs. The drugs used were: morphine hydrochloride purchased from SIFAC, Milan, Italy; naloxone hydrochloride, physostigmine sulphate, noradrenaline bitartrate, 3,4-dihydroxybenzylamine hydrobromide (internal standard for the noradrenaline determinations), acetylcholine chloride, hyoscine sulphate and guanethidine sulphate, all purchased from Sigma, St Louis, MO, USA.

Data analysis. The effects of naloxone on the various parameters were expressed as percentage variation with respect to the control values. Concentration-response relationships with 95% confidence limits were calculated by linear regression analysis according to Tallarida & Murray (1987). The activity of naloxone in different tests was evaluated by comparing equieffective concentrations in enhancing neurotransmitter release.

Results

Acetylcholine release. The mean resting acetylcholine release in normal and sympathetically denervated preparations was 23 ± 2 and 24 ± 1 ng g^{-1} min^{-1} (means $\pm$ s.e.m., n = 8), respectively. The mean electrically-evoked acetylcholine release was 56 ± 5 and 58 ± 2 ng g^{-1} min^{-1} (n = 8) in normal and sympathetically denervated preparations, respectively.

Naloxone dose-dependently increased resting and electrically-evoked acetylcholine release in normal and sympathetically denervated preparations (Fig. 1). Naloxone was more potent in increasing resting acetylcholine release in sympathetically denervated preparations. Concentrations enhancing resting acetylcholine release by 50% were 324 (158–659) and 72 (24–213) nM in normal and denervated preparations, respectively. No difference was observed in the concentrations enhancing by 50% electrically-evoked acetylcholine release in normal and denervated preparations (78 (18–347) and 66 (39–112) nM, respectively).

Noradrenaline release. The mean electrically-evoked noradrenaline release was $2{\cdot}3 \pm 0{\cdot}6$ ng g^{-1} min^{-1} (n = 13). It was dose-dependently enhanced by naloxone (Fig. 2). The concentration enhancing noradrenaline release by 50% was 6 (0·25–149) nM.

Electrical stimulation. In colonic preparations obtained from normal guinea-pigs, electrical stimulation of colonic longitudinally-mounted specimens evoked an excitatory response, which was unaffected by 1 μM naloxone (% variation = $-0{\cdot}3 \pm 1{\cdot}7$, n = 4).

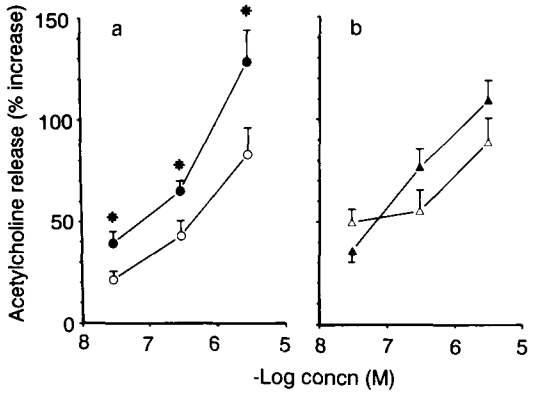

FIG. 1. Log concentration-effect relationships for naloxone in increasing resting (a) and stimulated (b) acetylcholine release in normal (open symbols) and denervated (closed symbols) preparations. Each point represents the mean of five experiments. Vertical bars indicate s.e.m. *$P < 0{\cdot}05$ vs normal preparations.

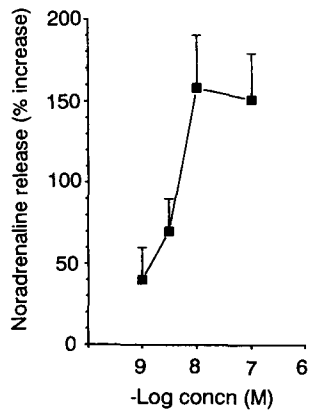

FIG. 2. Log concentration-effect relationship for naloxone in increasing noradrenaline release. Each point represents the mean of five experiments. Vertical bars indicate s.e.m.

Electrical stimulation of circular muscle strips in the presence of hyoscine and guanethidine, evoked NANC relaxations, which were enhanced by 1 μM naloxone. The increase in NANC relaxation was frequency-dependent, being 7 ± 1, 32 ± 7 and 50 ± 4% (n = 4) at 1, 2 and 4 Hz stimulation frequency, respectively.

Discussion

The present study, showing a facilitatory effect of naloxone on acetylcholine and noradrenaline release, substantiates the hypothesis that opioids exert a tonic restraint on neurotransmitter release in the guinea-pig distal colon.

In the enteric nervous system, both enkephalinergic and dynorphinergic neurons are widely distributed (Furness & Costa 1987) and are thought to modulate several gastrointestinal functions, including motility, acid and endocrine secretions, and fluid and electrolyte transport (Dockray 1987). However, it is difficult to predict the effects of opioids in a specific experimental model, since they depend on several factors such as the species, the gut level and the experimental conditions.

Inhibition of neurotransmitter release at cholinergic synapses (Paton 1957; Nakayama et al 1990) has been regarded as one of the major mechanisms by which opioids inhibit intestinal peristalsis. However, in some preparations, opioids can also cause contraction by inhibition of the non-adrenergic, non-cholinergic (NANC) inhibitory innervation (Shimo & Ishii 1978; Tonini et al 1985; Radomirov et al 1990) or by a direct muscular action (Kromer 1988; Bitar & Makhlouf 1982). Opioids have indeed been shown to modulate the release of both excitatory and inhibitory neurotransmitters such as substance P (Gintzler & Scalisi 1982), vasoactive intestinal polypeptide (Grider & Makhlouf 1987) and methionine-enkephalin (Glass et al 1986).

The observation that naloxone increases the release of acetylcholine (present data) and the efficiency of the peristaltic reflex (Marino et al 1992) is highly suggestive of a physiological role of opioids in modulating peristalsis in the guinea-pig colon. The increase in acetylcholine release observed with naloxone suggests that opioids tonically inhibit cholinergic synapses in the myenteric plexus. Furthermore, the fact that acetylcholine release was unchanged in sympathetically denervated animals is consistent with the hypothesis that, in the absence of the tonic adrenergic inhibition, other inhibitory systems may take control. The higher potency of naloxone in enhancing resting acetylcholine release after sympathetic denervation is consistent with this hypothesis. The present experiments also suggest that endoge-

nous opioids may tonically inhibit not only excitatory, but also adrenergic and NANC inhibitory pathways. Exogenous opioids have already been shown to inhibit catecholamine release from the guinea-pig ileum (Nakayama et al 1990) and the dog adrenal gland (Kimura et al 1988). In the latter experimental model, naloxone itself was able to enhance electrically-evoked catecholamine output. On the other hand, the lack of effect of naloxone on electrically-induced contractions in the longitudinal muscle seems to indicate that opioids do not exert a tonic restraint at this level. This is in keeping with the observation that morphine has no effect on electrically-induced contractions in the human taenia coli (Burleigh & Trout 1986).

The fact that opioids can inhibit noradrenaline release from adrenergic terminals in guinea-pig ileum (Nakayama et al 1990) taken together with the observation that the adrenergic agonist clonidine increases opioid peptide levels in the myenteric plexus (Schulz et al 1986) is consistent with the hypothesis of an interaction between enteric opioid neurons and adrenergic terminals, at least in the guinea-pig ileum (Colado & Martin 1992). The observed supersensitivity to morphine in inhibiting propulsion in the sympathetically denervated colon (Marino et al 1992) supports this hypothesis.

In conclusion, the present experiments provide evidence in favour of the existence of a tonic restraint exerted by endogenous opioids on excitatory (cholinergic) and inhibitory (adrenergic and NANC) neural pathways. They also suggest that, after suppression of the adrenergic inhibitory tone, the functional relevance of opioid inhibitory pathways is increased.

References

Bitar, K. N., Makhlouf, G. M. (1982) Specific opiate receptors on isolated mammalian gastric smooth muscle cells. Nature 297: 72–74

Burleigh, D. E., Trout, S. J. (1986) Morphine attenuates cholinergic nerve activity in human isolated colonic muscle. Br. J. Pharmacol. 88: 307–313

Colado, M. I., Martin, M. I. (1992) Effects of opioid and α_2-adrenoceptor agonists on the isolated ileum of morphine-dependent guinea-pigs during withdrawal and after clonidine treatment. J. Pharm. Pharmacol. 44: 101–104

Dockray, G. J. (1987) Physiology of enteric neuropeptides. In: Johnson, L. R. (ed.) Physiology of the Gastrointestinal Tract. Vol. 1, Raven Press, New York, pp 41–66

Frigo, G. M., Lecchini, S., Marcoli, M., Tonini, M., D'Angelo, L., Crema, A. (1984) Changes in the sensitivity to the inhibitory effects of adrenergic agonist on intestinal motor activity after chronic sympathetic denervation. Naunyn Schmiedebergs Arch. Pharmacol. 325: 145–152

Frigo, G. M., Galli, A., Lecchini, S., Marcoli, M. (1987) A facilitatory effect of bicuculline on the enteric neurones in the guinea-pig isolated colon. Br. J. Pharmacol. 90: 31–41

Furness, J. B., Costa, M. (1987) The Enteric Nervous System. Churchill Livingstone, London

Gatti, G., De Ponti, F., D'Angelo, L., Caravaggi, M., Lecchini, S., Frigo, G. M., Crema, A. (1987) A simple analytical method for determining catecholamine release from enteric neurons: an HPLC technique employing coulometric detection. Ital. J. Gastroenterol. 19: 282–284

Gintzler, A. R., Scalisi, J. (1982) Effects of opioids on non cholinergic excitatory response on the guinea-pig isolated ileum: inhibition of release of enteric substance P. Br. J. Pharmacol. 75: 199–205

Glass, J., Chan, W. C., Gintzler, A. R. (1986) Direct analysis of the release of methionine-enkephalin from guinea-pig myenteric plexus: modulation by endogenous opioids and exogenous morphine. J. Pharmacol. Exp. Ther. 239: 742–747

Grider, J. R., Makhlouf, G. M. (1987) Role of opioid neurons in the regulation of intestinal peristalsis. Am. J. Physiol. 253: G226-G231

Hjemdahl, P. (1984) Catecholamine measurements by high-performance liquid chromatography. Am. J. Physiol. 247: E13–E20

Hughes, J., Kosterlitz, H. W., Smith, T. W. (1977) The distribution of methionine-enkephalin and leucine-enkephalin in the brain and peripheral tissues. Br. J. Pharmacol. 61: 639–647

Kimura, T., Katoh, M., Satoh, S. (1988) Inhibition by opioid agonists and enhancement by antagonists of the release of catecholamines from the dog adrenal gland in response to splanchic nerve stimulation: evidence for the functional role of opioid receptors. J. Pharmacol. Exp. Ther. 244: 1098–1102

Kromer, W. (1988) Endogenous and exogenous opioids in the control of gastrointestinal motility and secretion. Pharmacol. Rev. 40: 121–156

Marcoli, M., Lecchini, S., De Ponti, F., Angelo, L., Crema, A., Frigo, G. M. (1985) Subsensitivity of enteric cholinergic neurones to α_2-adrenoceptor agonists after chronic sympathetic denervation. Naunyn Schmiedebergs Arch. Pharmacol. 329: 271–277

Marino, F., Marcoli, M., Lecchini, S., Frigo, G. M. (1992) Supersensitivity to morphine after chronic sympathetic denervation in guinea-pig colon. J. Pharm. Pharmacol. 44: 526–527

Mazzanti, L., Del Tacca, M., Breschi, M. C., Frigo, G. M., Friedmann, C., Crema, A. (1972) The time course of functional and morphological changes of the guinea-pig colon after "a frigore" denervation of the periarterial sympathetic nerves. Acta Neuropathol. (Berl) 22: 190–199

Nakayama, S., Taniyama, K., Matsuyama, S., Ohgushi, N., Tsunekawa, K., Tanaka, C. (1990) Regulatory role of enteric mu and kappa opioid receptors in the release of acetylcholine and norepinephrine from guinea-pig ileum. J. Pharmacol. Exp. Ther. 254: 792–798

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. Pharmacol. 11: 119–127

Paton, W. D. M., Vizi, E. S. (1969) The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea-pig ileum longitudinal muscle strip. Br. J. Pharmacol. 35: 10–28

Radomirov, R., Pencheva, N., Venkova, K., Davidoff, M. (1990) Effects of (met-5) enkephalin on the electrically-evoked mechanical responses in longitudinal and circular strips of the cat terminal ileum. Neuropeptides 17: 35–44

Roman, C., Gonella, J. (1987) Extrinsic control of digestive tract motility. In: Johnson, L. R. (ed.) Physiology of the Gastrointestinal Tract. 2nd edn, Raven Press, New York, pp 507–553

Schulz, R., Metzner, K., Dandekar, T., Gramsch, C. (1986) Opiates induce long-term increases in prodynorphin-derived peptide levels in the guinea-pig myenteric plexus. Naunyn Schmiedebergs Arch. Pharmacol. 333: 381–386

Shimo, Y., Ishii, T. (1978) Effects of morphine on non-adrenergic inhibitory responses of the guinea-pig taenia coli. J. Pharm. Pharmacol. 30: 596–597

Tallarida, R. J., Murray, R. B. (1987) Manual of Pharmacologic Calculations with Computer Programs. Springer-Verlag, New York, USA

Tonini, M., Onori, L., Perucca, E., Manzo, L., De Ponti, F., Crema, A. (1985) Depression by morphine of the excitability of intrinsic inhibitory neurons in the guinea-pig colon. Eur. J. Pharmacol. 115: 317–320