Effects of morphine on non-adrenergic inhibitory responses of the guinea-pig taenia coli

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Morphine or narcotic analgesics have been reported to inhibit noradrenaline release from adrenergic nerves in mouse vas deferens (Hughes, Kosterlitz & Leslie, 1975) and cat nictitating membrane (Henderson, Hughes & Kosterlitz, 1975). Recently high concentrations of enkephalins have been found in the longitudinal muscle-myenteric plexus of guinea-pig intestine by Hughes, Kosterlitz & Smith (1977) who proposed that the enkephalins function as neurotransmitter agents at morphine-sensitive synapses (Hughes, 1975; Kosterlitz & Hughes, 1975). However, morphine has no effect on the adrenergic inhibitory response in guinea-pig intestine (Henderson & others, 1975). The present report describes the effects of morphine on the electrically elicited relaxation of guinea-pig taenia coli, which is known to be innervated by two kinds of inhibitory nerves; adrenergic nerves and non-adrenergic nerves.

Male guinea pigs, 250–300 g, were killed by a blow on the head and perivascular nerve taenia preparations were prepared according to Burnstock, Campbell & Rand (1966). Each preparation was mounted in 50 ml organ bath containing Tyrode solution (mM) NaCl 136·9, KCl 2·7, $CaCl_2$ 1·8, NaH_2PO_4 0·4, $MgCl_2$ 1·0, $NaHCO_3$ 11·9, glucose 5·6, aerated with a mixture of 5% CO_2 in oxygen at 37°. A pair of stimulating electrodes, made of two platinum wire loops 3 mm apart, were placed around the mesenteric blood vessels for stimulation of the perivascular nerves and another pair of electrodes around the taenia and for its transmural stimulation. Perivascular nerve stimulation was with rectangular pulses of varied frequencies from 5 to 30 Hz, pulse duration 1 ms, maximal voltage, at 5 min intervals. Care was taken not to stimulate intramural non-adrenergic inhibitory nerves. Transmural stimulation of the taenia was also with rectangular pulses from 0·5 to 10 Hz, pulse duration of 0·3 ms maximal voltage, at 3 min intervals. The mechanical responses of the taenia were recorded with an isotonic transducer under a tension of 0·5 g. For the elimination of parasympathetic components in responses to the stimulation, the Tyrode solution contained 0·2 μM atropine sulphate. Drugs used were: morphine hydrochloride (Dainippon), naloxone hydrochloride (Endo Laboratories), guanethidine sulphate (Ciba), atropine sulphate (Wako) and tetrodotoxin (Sankyo). All drugs were dissolved in physiological saline (0·9% w/v NaCl).

The perivascular nerve stimulation of the taenia with the stimulus parameters described elicited only relaxation which depended on the stimulus frequency. The minimal and maximal relaxations were obtained at 5

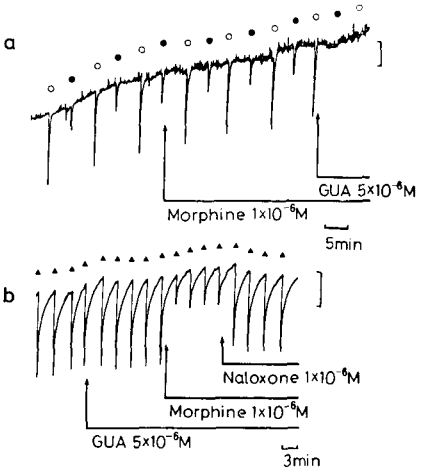

FIG. 1. Effects of morphine and naloxone on responses to perivascular nerve and transmural stimulations in the presence of atropine (2×10^{-7}M). (a) perivascular nerve was alternately stimulated at 10 Hz (●) and 30 Hz (○) with a train of 90 pulses at 5 min intervals. Morphine (1×10^{-6}M) did not cause any depression of the inhibitory response to perivascular nerve stimulation. (b) Taenia was stimulated at 0·5 Hz (▲) for 4 s every 3 min in the presence of guanethidine (GUA, 5×10^{-6} M). Morphine (1×10^{-6}M) decreased the inhibitory response to transmural stimulation of the taenia. The inhibition by morphine was reversed by naloxone (1×10^{-6}M). Vertical bar indicates 0·5 mm.

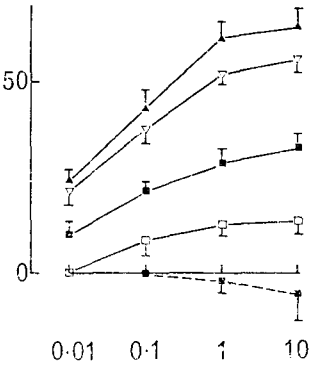

FIG. 2. Effects of morphine on responses to perivascular nerve stimulation (PS) in the presence of atropine (2×10^{-7}M) and transmural stimulation (TS) in the presence of atropine and guanethidine (5×10^{-6}M). ■ - - - - - ■ PS, 5 Hz, 90 pulses, n = 6. ▲——▲ TS, 0·5 Hz, 4 s, n = 10. ▽——▽ TS, 1 Hz, 4 s, n = 20. ■——■ TS, 5 Hz, 4 s, n = 9. □——□ TS, 10 Hz, 4 s, n = 4. Ordinate: inhibition of relaxation as a percentage of the initial response. Values are mean ± s.e.m. Abscissa: morphine concentration (μM).

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and 30 Hz respectively. These relaxations seemed to be mostly due to the stimulation of adrenergic nerve, since they were almost completely abolished by tetrodotoxin (5×10^{-7}M) or guanethidine (5×10^{-6}M). Morphine at less than 1×10^{-6}M did not depress the elicited relaxation (Fig. 1a). At a higher dose (1×10^{-5}M), the drug did not inhibit the relaxation elicited with 5 Hz, rather it was potentiated slightly (Fig. 2). This potentiation may be caused by inhibition of neuronal uptake of transmitter (Starke, 1977). Transmural stimulation in the presence of guanethidine (5×10^{-6}M) elicited only relaxation which was abolished by tetrodotoxin (5×10^{-7}M).

It is suggested that the relaxation elicited is mainly due to the stimulation of non-adrenergic inhibitory nerves. Morphine reversibly dose-dependently depressed these elicited relaxations (Fig. 1b). This inhibitory effect of morphine varied inversely with stimulus frequency (Fig. 2). At 0·5 Hz for 4 s, the drug inhibited the elicited response by about 60% while at 10 Hz its action was negligible (Fig. 2). Naloxone (1×10^{-6}M), a pure opiate antagonist (Kosterlitz & Watt, 1968), almost completely reversed the inhibition of morphine (Fig, 1b).

In conclusion, morphine (1×10^{-8}—1×10^{-6}M) has no inhibitory effect on the adrenergic inhibitory response of the taenia to perivascular stimulation. On the other hand, it depresses the non-adrenergic inhibitory response of the taenia to transmural stimulation via activation of opiate receptors probably located in the myenteric plexus of the taenia. The depression is negatively correlated with the frequency of nerve stimulation.

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REFERENCES

BURNSTOCK, G., CAMPBELL, G. & RAND, M. J. (1966). *J. Physiol.*, **182**, 504–526.
HENDERSON, G., HUGHES, J. & KOSTERLITZ, H. W. (1975). *Br. J. Pharmac.*, **53**, 505–512.
HUGHES, J. (1975). *Brain Res.*, **88**, 295–308.
HUGHES, J., KOSTERLITZ, H. W. & LESLIE, F. M. (1975). *Br. J. Pharmac.*, **53**, 371.
HUGHES, J., KOSTERLITZ, H. W. & SMITH, T. W. (1977). *Ibid.*, **61**, 639–647.
KOSTERLITZ, H. W. & HUGHES, J. (1975). *Life Sci.*, **17**, 91–96.
KOSTERLITZ, H. W. & WATT, A. J. (1968). *Br. J. Pharmac.*, **33**, 266–276.
STARKE, K. (1977). *Rev. Physiol. Biochem. Pharmac.*, **77**, 2–99.

The relative activity of prostacyclin (PGI_2) and a stable analogue 6β-PGI_1 on the gastrointestinal and cardiovascular systems

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Prostacyclin (PGI_2), a major product of arachidonic acid metabolism in vascular tissue, is a potent but unstable vasodilator and inhibitor of platelet aggregation (Moncada, Gryglewski & others, 1976). More recently, prostacyclin has been shown to be generated by the gastric mucosa of several species (Moncada, Salmon & others, 1978), and to be a potent inhibitor of gastric acid secretion and erosion formation in the gastric mucosa of the rat (Whittle, Boughton-Smith & others, 1978). We now describe the activity of a stable 5-6-dihydro prostacyclin, 6β-PGI_1 (Johnson, Lincoln & others, 1977) on some aspects of gastrointestinal function and the cardiovascular system.

Inhibition of gastric acid secretion and concomitant changes in systemic arterial blood pressure in the urethane-anaesthetized rat were determined as previously described (Main & Whittle, 1973). During steady submaximal rates of acid secretion induced by pentagastrin (0·5 μg kg^{-1} min^{-1}, i.v.), prostacyclin, its chemical decomposition product, 6-oxo-$PGF_{1\alpha}$ (Johnson & others, 1976) or 6β-PGI_1, each dissolved in an isotonic sodium bicarbonate solution (1·25% w/v; pH 8·6; 0°) were infused intravenously. The fall in acid output, which reached stable levels within 30 min was expressed as % inhibition of the control secretory values (1·5–2·5 μequiv min^{-1}). As is shown in Fig. 1, the stable analogue was some 16 times less potent than prostacyclin on intravenous infusion. Like prostacyclin, 6β-PGI_1 lowered systemic arterial blood pressure (BP) (Fig. 1). For doses inhibiting acid output by 50% (ID50), 6-oxo-$PGF_{1\alpha}$ and prostacyclin have similar relative activities in inhibiting gastric acid secretion and in reducing BP, whereas the analogue was relatively more active as an antisecretory agent (Table 1). Thus, the stable analogue shows some selectivity of action towards the gastric antisecretory actions but away from the cardiovascular actions of prostacyclin.

In the studies on the isolated lumen-perfused whole-stomach of the immature (30–50 g) rat (Bunce & Parsons, 1976), prostaglandins were added to the

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