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Nerve-mediated non-adrenergic inhibitory responses of guinea-pig taenia caeci: further evidence of depression by morphine

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Morphine is known to inhibit the electrically-induced contractile response of guinea-pig ileum (Paton 1957; Gyang & Kosterlitz 1966), guinea-pig oesophagus (Kamikawa & Shimo 1983), cat nictitating membrane (Henderson et al 1975) and mouse vas deferens (Henderson et al 1972; Hughes et al 1975) by inhibiting the release of acetylcholine or noradrenaline from nerves. Recently, we found that the nerve mediated non-adrenergic inhibitory response of the guinea-pig taenia caecum to electrical stimulation was inhibited by morphine or opioid peptides (Shimo & Ishii 1978; Ishii 1981) and potentiated by naloxone (Ishii 1981). These observations were later confirmed by Muramatsu et al (1979) and Leander et al (1981). However, Small & Yong (1983) failed to detect our reported action of morphine on the same inhibitory response.

In the present report, we discuss the reason for the difference between our results and those of Small & Yong (1983), and present further evidence of the ability of morphine to inhibit the non-adrenergic inhibitory response using the ganglion-stimulating drugs, nicotine and 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) which are known to stimulate the non-adrenergic nerve cell bodies in the myenteric plexus of the guinea-pig taenia caecum (Burnstock et al 1966).

Materials and methods

Male albino (Dunkin Hartley strain) or tricoloured guinea-pigs, 250–400 g, were killed by a blow on the head. Taenia strip preparations (15 mm long) attached to the myenteric plexus were excised from the caecum at about 2–3 cm distant from the ileo-caecal junction. The excised preparation was immersed in a 10 ml organ bath filled with Tyrode solution of the following composition (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 (pH 7·4). This Tyrode solution always contained atropine (2×10^{-7} M) and guanethidine (5×10^{-6} M) for the elimination of cholinergic and adrenergic responses and was bubbled with a mixture of 95% O_2 and 5% CO_2 and maintained at 37 °C. The preparation was suspended vertically under 0·3–0·5 g load and allowed to equilibrate for at least 60 min before the start of experiments. During the equilibration period, the bathing medium was replaced every 20 min. Responses of the taenia were recorded on an inkwriting recticorder (Nihon Kohden, RJQ-3006) using an isotonic transducer (ME Commercial, ME-4012). Electrical stimulation of nerves in the taenia was achieved by means of two platinum ring electrodes (4 mm internal diameter, 3 mm apart) placed around the tissue and unipolar rectangular pulses (0·3 ms pulse duration for 4 s at 0·5 Hz) were applied at intervals of 3 min. These electrode design and stimulus parameters were as described by Shimo & Ishii (1978) except that various current strengths were used in the present studies. When the current provided by the electric stimulator (Nihon Kohden, SEN-3201) was varied from 95 to 350 mA, the voltage strength between the electrodes was displayed on a oscilloscope (Nihon Kohden, VC-8) and varied from 3·7 to 13·5 V.

The effects of drugs on the elicited relaxations were measured as the percentage changes of the control relaxation obtained before the drugs were applied to the bath. The data obtained are expressed as mean ± s.e. mean. Significance was calculated using paired Student's *t*-test.

Drugs used were morphine hydrochloride (Dainippon), naloxone hydrochloride (Endo Laboratories), nicotine tartrate, hexamethonium chloride, atropine sulphate (Wako Pure Chem.), tetrodotoxin (Sankyo), 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), (−)isoprenaline hydrochloride (Sigma) and guanethidine sulphate (Ciba). To prepare stock solutions, all drugs were dissolved in physiological saline (0·9% w/v NaCl). The molar concentrations of drugs described in this paper refer to the final bath concentration.

Results

Inhibitory effects of morphine and tetrodotoxin (TTX) on the electrically-induced relaxations. The isolated taenia caecum of the guinea-pig usually developed tone during the incubation period of 60 min. In the presence of atropine (2×10^{-7} M) and guanethidine (5×10^{-6} M), electrical field stimulations with various current strengths (95–350 mA) produced relaxations of the taenia which were sometimes followed by a secondary contraction. These elicited relaxations tended to level off when the stimulus strength rose to 195 or 220 mA (voltage between the electrodes: 7·6 or 8·6 V, respectively). However, a further increase in the strength of applied current caused the amplitude of the relaxations to increase again. Thus, no well defined 'maximal' or

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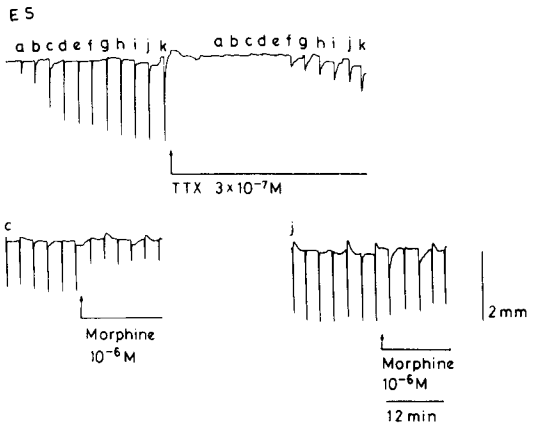

FIG. 1. Inhibitory effects of tetrodotoxin (TTX 3×10^{-7} M, upper trace) and morphine (10^{-6} M, lower trace) on the relaxations of the guinea-pig taenia caecum induced by electrical field stimulations (0·5 Hz, 0·3 ms, 4 s and 95–350 mA) at 3 min intervals in the presence of atropine (2×10^{-7} M) and guanethidine (5×10^{-6} M). ES = electrical field stimulation. a = current strength of 95 mA; b (120 mA); c (145 mA); d (170 mA); e (195 mA); f (220 mA); g (245 mA); h (270 mA); i (295 mA); j (325 mA); k (350 mA). The same preparation was used for TTX and morphine. Vertical calibration shows 2 mm length change of the tissue.

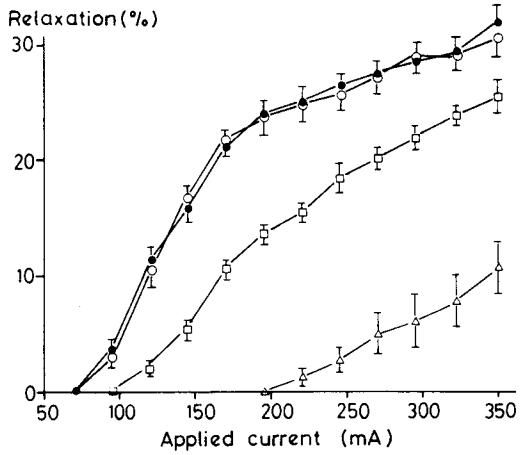

FIG. 2. Inhibitory effects of morphine and tetrodotoxin on the relaxations of the guinea-pig taenia caecum induced by electrical field stimulations (0·5 Hz, 0·3 ms, 4 s and 95–350 mA) and the antagonism of morphine's action by naloxone. (●) Control; (□) in the presence of morphine (10^{-6} M); (○) in the presence of morphine (10^{-6} M) and naloxone (10^{-6} M); (△) in the presence of morphine (10^{-6} M), naloxone (10^{-6} M) and tetrodotoxin (3×10^{-7} M). Atropine (2×10^{-7} M) and guanethidine (5×10^{-6} M) were present throughout the experiment. Each curve was obtained after pooling results from the taeniae of the albino and tricoloured guinea-pigs, since neither qualitative nor quantitative difference between these strips were observed. Each point is the mean value from 9 preparations and vertical bars represent s.e. mean. Abscissa scale, applied current (mA). Ordinate scale, % maximal relaxation induced by isoprenaline (10^{-6} M).

'supramaximal' strength of stimulation was obtained within the range tested (Fig. 1, 2).

Morphine (10^{-6} M) significantly inhibited ($P < 0.01$, paired t-test) all the elicited relaxations, but the degree of inhibition was reduced by increasing the stimulus strength. The relaxation of the taenia to electrical field stimulation at 145 mA was inhibited by 67·3 ± 4·7%, while that at 325 mA was inhibited only by 18·2 ± 3·4% (Fig. 1, 2). These inhibitory actions of morphine (10^{-6} M) were completely reversed by an opiate antagonist, naloxone (10^{-6} M) (Fig. 2). On the other hand, tetrodotoxin (TTX, 3×10^{-7} M) completely abolished the relaxations of the taenia to lower current stimulations (95–195 mA), but could not abolish those to higher current stimulations (220–350 mA) (Fig. 2). The relaxations which were abolished by TTX are due to a stimulation of the non-adrenergic inhibitory nerves, since TTX had been shown to block nerve conduction by inhibiting sodium influx across the neuronal membrane (Kao 1966).

Inhibitory effects of morphine and TTX on the nicotine- and DMPP-induced relaxations. As shown in Fig. 3, nicotine (3×10^{-5} M) in the presence of atropine (2×10^{-7} M) and guanethidine (5×10^{-6} M) produced a transient relaxation equivalent to 36·6 ± 5·4% (n = 16) of the response of isoprenaline (10^{-6} M). Pretreatment with hexamethonium (10^{-4} M) or TTX (3×10^{-7} 7M) completely inhibited this nicotine-induced relaxation. Therefore, this response may be also due to a stimulation of the non-adrenergic inhibitory neurons with their cell bodies in the myenteric plexus. These results are in accordance with those of Burnstock et al (1966).

Morphine reversibly inhibited the nicotine-induced relaxation, in a concentration-dependent manner, and average inhibitions at 0·01, 0·1 and 1 μM were 31·7 ± 8·6 (n = 7). 57·2 ± 5·1 (n = 6) and 84·1 ± 4·1% (n = 11), respectively. The inhibitory action of morphine (10^{-6} M) was completely antagonized by the addition of naloxone (10^{-6} M) (Fig. 3). Similar results were also obtained with DMPP (3×10^{-5} M).

Discussion

In our previous report, we showed that morphine or opioid peptides could inhibit the relaxation of the taenia to non-adrenergic intramural nerve stimulation (0·3 ms pulse duration, 0·5 Hz and 7·6–8·6 V) and this inhibition was inversely related to the stimulus frequency (Shimo & Ishii 1978; Ishii 1981). Small & Yong (1983), however, could not confirm this inhibitory action of morphine, even at a lower stimulation frequency (0·3 ms pulse duration, 0·02–1 Hz).

The explanation for this discrepancy is not that Small & Yong (1983) used animals whose taeniae were

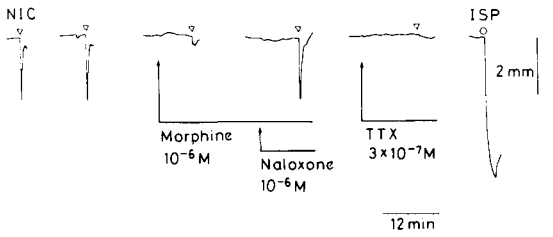

FIG. 3. Inhibitory effects of morphine (10^{-6} M) and tetrodotoxin (TTX 3×10^{-7} M) on the nicotine-induced relaxation in the presence of atropine (2×10^{-7} M) and guanethidine (5×10^{-6} M), and an antagonism of morphine's action by naloxone (10^{-6} M). Nicotine (NIC, 3×10^{-5} M) was added to the bathing medium at 30 min intervals. ISP, isoprenaline 10^{-6} M.

uniquely insensitive to morphine. We have used the same albino guinea-pigs (Dunkin Hartley strain) as they used. Furthermore, our results with morphine have been confirmed by Muramatsu et al (1979) and with opioid peptides by Leander et al (1981).

The difference between our results and those of Small & Yong (1983) may be due to a methodological difference. Small & Yong (1983) would probably use a strong stimulus current to stimulate the non-adrenergic inhibitory nerves of the taenia. This is supported by the following observations. (1) The relaxations of the taenia to electrical field stimulations (0·5–5 Hz) which Shimo & Ishii (1978), Muramatsu et al (1979), Ishii (1981) and Leander et al (1981) demonstrated were all completely abolished by TTX (10^{-7}–10^{-6} M), while the elicited relaxation (1 Hz) demonstrated by Small & Yong (1983) was not abolished by TTX (3×10^{-7} M), leaving some residual relaxations. (2) In our present experiments, the relaxations of the taenia to lower current stimulations (0·5 Hz, 95–195 mA) were completely abolished by TTX (3×10^{-7} M), while those to higher current stimulations (0·5 Hz, 220–350 mA) were not abolished by TTX (3×10^{-7} M). These TTX insensitive relaxations were resistant to morphine.

This antagonism of morphine's action by increasing the stimulus current has also been reported in the cholinergic system using the myenteric plexus-longitudinal muscle preparation of the guinea-pig. Sawynok & Jhamandas (1976), who have compared the effects of submaximal and supramaximal stimulation, have found that morphine markedly inhibited the electrically induced contractile response when submaximal electrical stimulation of 0·1 Hz which produced a twich height of about 80% of maximal response was used. Furthermore, North & Tonini (1977) have shown that the inhibitory action of normorphine on the electrically induced twich contraction could be overcome by increasing the stimulus current, even though the stimulus current was already supramaximal in the absence of normorphine.

Further evidence for the inhibitory action of morphine in the guinea-pig taenia caecum was obtained from experiments with ganglion-stimulating drugs, nicotine and DMPP. That is, the relaxations of the taenia to both nicotine (3×10^{-5} M) and DMPP (3×10^{-5} M) which were able to stimulate the non-adrenergic nerve cell bodies in the myenteric plexus were markedly inhibited by morphine (10^{-6} M). These inhibitory actions by morphine were also completely reversed by naloxone (10^{-6} M).

In conclusion, morphine can inhibit the non-adrenergic inhibitory neurotransmission of the guinea-pig taenia caecum, via opiate receptors probably located in the neurons. The difference between our previous results and those of Small & Yong may be due to a difference of current strength used for electrical field stimulation of the taenia. The stimulus currents they used may have been too strong to demonstrate the inhibitory effect of morphine on the non-adrenergic inhibitory response of the taenia caecum.

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