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ENKEPHALIN RECEPTOR IN THE RABBIT ILEUM

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The effects of the classical morphine-like compounds and opioid peptides on the electrically-induced contractions of the myenteric plexus-longitudinal muscle strip of the rabbit ileum were studied. The results indicate that the rabbit ileum contains the new type of opiate receptor, which is not sensitive to morphine but is sensitive to enkephalins, and provides evidence that the enkephalins may function as neurotransmitters in the intestine. The action of the enkephalins was antagonized by relatively high dose of naloxone.

Introduction It is well-known that the guinea-pig ileum contains opiate receptors and it has been widely employed as a suitable preparation for the measurement of the activity of the classical morphine-like compounds (Paton & Zar, 1968) and the newly discovered endogenous opioid peptides (Waterfield, Smokcum, Hughes, Kosterlitz & Henderson, 1977). In contrast, the rabbit ileum was found to be insensitive to morphine (Cowie, Kosterlitz & Waterfield, 1978), although the presence of enkephalins in the rabbit intestine has been demonstrated (Hughes, Kosterlitz & Smith, 1977). Thus, the significance of enkephalins in the rabbit intestine, in which receptors for enkephalins have not been demonstrated, is uncertain at present. After evidence for the existence of multiple opiate receptors had been obtained from the several lines of investigation (Martin, Eades, Thompson, Huppler & Gilbert, 1976; Lord, Waterfield, Hughes & Kosterlitz, 1977), it became interesting to investigate the action of opioid peptides on the rabbit intestine.

Methods The rabbit ileum was used after approximately 30 cm of ileum nearest to the ileo-caecal junction had been discarded. The myenteric plexus-longitudinal muscle strip was prepared as described by Paton & Zar (1968). The strip, approximately 2 cm long, was suspended in a 4 ml organ bath, which contained Krebs solution and 20 μM choline chloride, and was kept at 36°C and bubbled with 95% O_2 and 5% CO_2. The resting tension of the strip was maintained at 1 g. After both tone and spontaneous rhythmic contractions became stable, the strip was stimulated through two platinum ring electrodes with supramaximal rectangular pulses of 0.5 ms duration as described in the legend of Figure 1. Both spontaneous rhythmic contractions and electrically induced twitch-like contractions of the strip were recorded isometrically by means of a strain gauge transducer and an ink-writing pen oscillograph.

Results The effects of opioid peptides on the electrically evoked contractions of the strip are shown in Figure 1. Both [Met^5]-enkephalin and [Leu^5]-enkephalin, which have been shown to be present in the intestine (Hughes *et al.*, 1977), significantly depressed the contractions of the strip. The potency of these two pentapeptides was shown to be essentially the same. The synthetic enkephalin analogues, [D-Ala^2,Met^5]-enkephalin, [D-Ala^2,Leu^5]-enkephalin and [D-Ala^2,Met^5]-enkephalinamide, were more potent in inhibiting the twitch-like contractions of the strip than naturally occurring enkephalins. Another endogenous opioid peptide, β-endorphin, which has not been detected in the intestine (Hughes *et al.*, 1977), was less potent in inhibiting the contractions of the strip than enkephalins. The inhibitory action of [Met^5]-enkephalin on the contractions of the strip was antagonized by naloxone with a K_e (equilibrium dissociation constant) value of 43 nM, which was determined by the 'single dose' method of Kosterlitz & Watt (1968). The inhibitory actions of opioid peptides other than [Met^5]-enkephalin were also completely reversed by naloxone at concentrations approximately ten fold higher than those of the agonist peptide. Naloxone itself had no effect on the twitch-like contractions of the strip.

In contrast to opioid peptides, morphine at concentrations ranging from 10^{-7} to 10^{-5} M did not produce significant inhibition of the electrically-evoked contractions of the strip as found previously (Cowie *et al.*, 1978). The other classical, non-peptide narcotic analgesics such as methadone and pethidine at concentrations ranging from 10^{-7} to 10^{-6} M also showed no significant depression of contractions of the strip. On the other hand, the electrically-induced contractions of the strip were inhibited by atropine; the concentration causing 50% inhibition of contractions was 190 nM.

Discussion The opiate receptor in the rabbit ileum was shown in the present investigation to have the following different characteristics from those already reported: (1) The classical non-peptide narcotic analgesics such as morphine, pethidine, and methadone at

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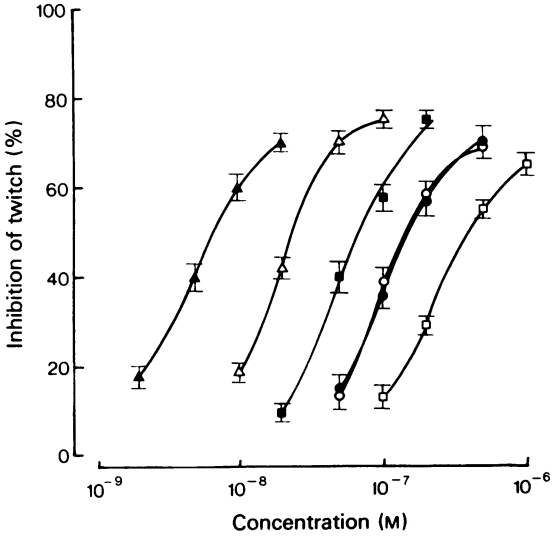

Figure 1 Dose-response curves for the inhibitory effects of opioid peptides on the electrically induced twitch-like contractions of the myenteric plexus-longitudinal muscle preparation of the rabbit ileum. By watching the spontaneous contractions on an ink writing pen oscillograph, electrical stimulation was applied manually (at a frequency ranging from 0·07 to 0.09 Hz depending on the frequency of spontaneous rhythmic contractions) at the point immediately before the maximal relaxation of the strip in the ratio of three spontaneous contractions to one stimulation. By this procedure, approximately the same magnitude of twitch-like contractions was obtained after each stimulation. The distance from the top of the twitch-like contraction to the top of the spontaneous contraction immediately before the twitch-like contraction was regarded as the height of the electrically evoked contractions. The average value of the height of seven twitch-like contractions in the absence and the presence of a peptide was calculated to obtain the percentage inhibition. To obtain the value in the presence of a peptide, the height of seven consecutive contractions was measured after the maximum depressant effect was obtained. Abscissa scale, concentration of opioid peptides (M); ordinate scale, inhibition of twitch-like contractions (%). Each point is the mean of four to seven observations, on tissues from different animals. Vertical bars show standard errors. (▲) [D-Ala^2,Met^5]-enkephalin; (△) [D-Ala^2,Leu^5]-enkephalin; (■)[D-Ala^2,Met^5]-enkephalinamide; (□) β-endorphin; (●) [Met^5]-enkephalin; (○) [Leu^5]-enkephalin.

concentrations ranging from 10^{-7} to 10^{-6} M have essentially no inhibitory action on the rabbit ileum while these agents have significant effects on the other peripheral neuro-effector junctions such as guinea-pig ileum (Paton & Zar, 1968) and mouse vas deferens (Henderson, Hughes & Kosterlitz, 1972), and on the μ and κ receptors in the chronic spinal dog (Martin *et al.*, 1976). (2) Naturally occurring enkephalins are more potent than β-endorphin in the rabbit ileum while the latter is more potent than the former in the rat vas deferens, in which both classical narcotic analgesics and naturally occurring enkephalins have low potency (Lemaire, Magnan & Regoli, 1978). (3) The K_e value of naloxone against [Met^5]-enkephalin in the rabbit ileum was approximately twenty and two fold higher than that found in the guinea-pig ileum and in the mouse vas deferens, respectively (Waterfield *et al.*, 1977).

The fact that the electrically-induced contractions of the strip were inhibited by atropine suggests that opioid peptides depress the twitch-like contractions by inhibiting the electrically-evoked release of acetylcholine in the rabbit ileum as well as the guinea-pig ileum, in which inhibition of acetylcholine release by opioids is well established (Paton & Zar, 1968; Cowie *et al.*, 1978).

Since these preliminary experiments showed that the electrically evoked contractions of the intestine of mice and rats, were also not significantly depressed by morphine but inhibited by enkephalins, it is suggested that the intestinal opiate receptor may generally have similar characteristics to that of rabbit ileum.

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References

COWIE, A.L., KOSTERLITZ, H.W. & WATERFIELD, A.A. (1978). Factors influencing the release of acetylcholine from the myenteric plexus of the ileum of the guinea-pig and rabbit. *Br. J. Pharmac.*, **64**, 565–580.

HENDERSON, G., HUGHES, J. & KOSTERLITZ, H.W. (1972). A new example of a morphine-sensitive neuro-effector junction: adrenergic transmission in the mouse vas deferens. *Br. J. Pharmac.*, **46**, 764–766.

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. Pharmac.*, **61**, 639–647.

KOSTERLITZ, H.W. & WATT, A.J. (1968). Kinetic parameters of narcotic agonists and antagonists, with particular reference to N-allylnoroxymorphone (naloxone). *Br. J. Pharmac.*, **33**, 266–276.

LEMAIRE, S., MAGNAN, J. & REGOLI, D. (1978). Rat vas deferens: a specific bioassay for endogenous opioid peptides. *Br. J. Pharmac.*, **64**, 327–329.

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

MARTIN, W.R., EADES, C.G., THOMPSON, J.A., HUPPLER, R.E. & GILBERT, P.E. (1976). The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. *J. Pharmac. exp. Ther.*, **197**, 517–532.

PATON, W.D.M. & ZAR, M.A. (1968). The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle strips. *J. Physiol.*, **194**, 13–33.

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

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