chloroquine to potentiate oedema in adjuvant arthritis (Stone et al 1975).

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Inhibition by enkephalins of peristaltic activity of the rabbit ileum and its reversal by naloxone

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Two pentapeptides, the enkephalins have been isolated and identified from the mammalian gastrointestinal tract and have been shown to be potent opiate agonists (Hughes 1975; Lord et al 1977; Hughes et al 1977). Further work has demonstrated that methionine (met-)-enkephalin and D-Ala^2-Leu-enkephalinamide affected the peristaltic reflex of the guinea-pig isolated ileum (van Nueten et al 1977; Kromer et al 1980). On the other hand, the rabbit isolated ileum has been scarcely used as model to study the peristaltic reflex (Feldberg & Lin 1949; Beleslin et al 1978). However, after evidence of the existence of met- and leucine (leu)-enkephalin as well as of enkephalin receptors in the rabbit intestine was reported (Hughes et al 1977; Oka 1980) it became interesting to investigate the action of enkephalins on the peristaltic activity of rabbit isolated ileum.

Method

In these experiments 16 rabbits of either sex, 2–2·5 kg were used. The peristaltic reflex was studied in the isolated ileum, by means of a modified Trendelenburg method (Beleslin et al 1978). Briefly, ileal segments were suspended in Tyrode solution at 37 °C and at pH 7·3 gassed with 95% O_2 and 5% CO_2 in 20 ml organ bath. The intraluminal pressure changes were recorded by means of a float recorder (Stephenson 1948), the volume of fluid expelled was recorded by means of a drop recording unit (silver drop tube, electronic drop recording unit, Thorp impulse counter and time clock) and measured by a measuring cylinder, while the movements of the longitudinal muscle were recorded via an isotonic frontal lever. After both tone and spontaneous rhythmic contractions became stable, the peristaltic reflex was elicited by increasing the intraluminal pressure by 1·5 to 2·5 cm H_2O maintained for the duration of experiment. The organ bath fluid was renewed every 10 min. All substances were added to the bath and therefore acted from the serosal surface of the ileum. The concentrations of enkephalin refer to the peptides, while those of naloxone to naloxone hydrochloride.

When the rabbit isolated ileum is kept under a constant intraluminal pressure of a few cm of H_2O for several hours the peristaltic waves are often frequent during the first 10 to 20 min of an experiment, becoming gradually slower and more regular in most preparations throughout the experiment. When this activity appears, the ileal segment is suitable for studying the effect of drugs that depress the propulsive movements of the intestine.

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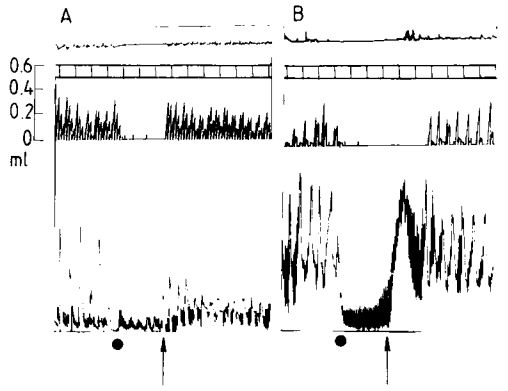

FIG. 1. Rabbit isolated ileum subjected to constant intraluminal pressure of 2 cm H_2O. The intraluminal pressure changes are shown in the upper record, increase in pressure upwards; the trace shown in the middle of the record represents the expelled fluid in ml, expulsion upwards; the movements of the longitudinal muscle are shown in the lower record, contractions upwards. At the dot in A met-enkephalin (150 nmol ml^{-1}) and at the arrow naloxone (100 nmol ml^{-1}) were added into the organ bath. B was taken 30 min after washing out met-enkephalin and naloxone. At the dot in B leu-enkephalin (150 nmol ml^{-1}) and at the arrow naloxone (10 nmol ml^{-1}) were added into the organ bath. Time marker in minutes.

Results

In 5 experiments met-enkephalin (50–150 nmol ml^{-1}) abolished the peristaltic reflex of the rabbit isolated ileum. In 5 similar experiments, leu-enkephalin (50–150 nmol ml^{-1}) was as effective as met-enkephalin. Such an experiment is shown in Fig. 1A and B; both peptides in concentrations of 150 nmol ml^{-1} relaxed the longitudinal muscle and abolished the propulsive activity a few seconds after the addition to the organ bath. The type of pendular movements after leu-enkephalin resembled the type of rhythmic activity when the intraluminal pressure was zero or below. When met-enkephalin (3 experiments) and leu-enkephalin (3 experiments) were used in concentrations from 3 to 50 nmol ml^{-1} the peristaltic activity was only depressed. The addition of naloxone (5–150 nmol ml^{-1}) restored the peristaltic activity previously blocked by met- (5 experiments) and leu-enkephalin (5 experiments). However, when naloxone was used in the smallest concentrations of 5 to 20 nmol ml^{-1} the propulsive activity and the peristaltic waves appeared only after a latent period of several minutes (6 experiments). As shown in Fig. 1A, naloxone in a concentration of 100 nmol ml^{-1}, almost immediately after the addition, restored the peristaltic waves as well as the propulsive activity, while in the small concentration of 10 nmol ml^{-1}, naloxone restored the peristaltic activity only after a latent period of about 2 min. During this time the relaxation disappeared and the tone of the longitudinal muscle gradually reached its normal level (Fig. 1B).

Discussion

The present results demonstrate that the peristaltic activity of the rabbit isolated ileum is sensitive to the inhibitory effect of enkephalins. Similar findings have been reported for the inhibition by met-enkephalin of peristaltic activity in the guinea-pig isolated ileum (van Nueten et al 1977). The effect of enkephalins on the peristaltic activity of rabbit isolated ileum occurred at rather low concentrations of the peptides. This action appeared to be mediated by opioid-like receptors because it was reversed by naloxone. The observation that the antagonism occurred at low antagonist concentrations considerably strengthens this interpretation. The presence of opioid-like receptors in the rabbit ileum has already been demonstrated (Oka 1980). In this connection, electrically-induced contractions of the myenteric plexus-longitudinal muscle strip of rabbit ileum are not sensitive to morphine and classical morphine-like compounds (Cowie et al 1978). In conclusion, the present experiments provide further evidence that enkephalins are not only involved in the modulation of contractile activity, but also in the transmission or modulation of peristaltic activity in the rabbit ileum.

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