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Release of Endogenous Opioid Peptides in Guinea-pig Duodenum and Ileum

In 1975, HUGHES *et al.* (3) reported the isolation of enkephalins from the guinea-pig ileum and in 1976 ELDE *et al.* (1) localised this pentapeptide immunohistochemically in the myenteric plexus.

The distribution of these peptides is not uniform throughout the gastrointestinal tract of the guinea-pig, and highest concentrations occurring in the duodenum (2).

Previous work (6) has demonstrated that an electrical stimulation at 10 Hz of the guinea-pig ileum myenteric plexus longitudinal muscle (MPLM) preparation, elicits an inhibitory response (IR) which is mediated by endogenous opioid release.

In the present work, we have studied comparatively the effect of an electrical stimulation at 10 Hz, on the MPLM guinea-pig duodenum and ileum in order to determine by an indirect way the possible differences in the endogenous opioid release at different levels of the small intestine.

The experiments were carried out on the guinea-pig ileum and duodenum MPLM preparations, which were prepared by a combination of the procedures described by PATON and ZAR (5) and KOSTERLITZ *et al.* (4). Each strip of tissue was then suspended in a 2 ml organ bath containing Krebs bicarbonate solution at 37° C and gassed with 95 % O_2 - 5 % CO_2.

The strips were stimulated at a frequency of 0.125 Hz (basal frequency of stimulation), supramaximal voltage (40 v) and duration 1 ms.

In order to produce an opiate like inhibition of the contractions, we increased the frequency of stimulation to 10 Hz for 15 s, after which the basal frequency of stimulation was resumed. Periods of stimulation at 10 Hz for 30 s were repeated every 30 min for 6 periods the last of which was performed with naloxone (10^{-7}M) added directly to the organ bath 5 min before the stimulation of 10 Hz.

The inhibitory response (IR) was calculated by measuring the area of the electrically induced contractions 5 min before (basal response, BR) and 5 min after (poststimulation response, PSR), the stimulation at 10 Hz.

The formula used for the calculation is

$$IR = (BR\text{-}PSR/BR) \times 100$$

The reversal (R) produced by naloxone is the percentage of the IR that is reversed by the drug and it is calculated as follows: $I = IRc - IRa/IRc \times 100$ where IRc is the IR in control conditions and IRa is the IR in presence of the antagonist.

The IR was 48 ± 4.5 % (n = 6) in strips obtained from duodenum and 29 ± 2.2 % (n = 6) from ileum guinea-pig. In the presence of naloxone the R was 79 ± 4.2 (n = 6) and 59 ± 2.4 % (n = 6) respectively.

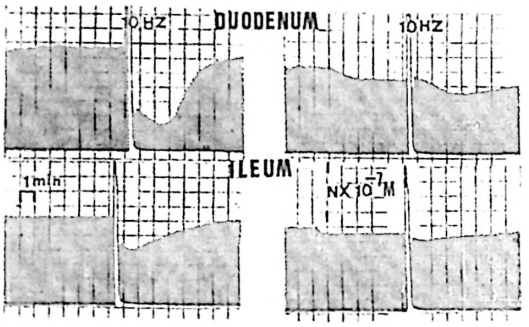

Fig. 1. *Inhibition of the twitch response of the MPLM preparation, by stimulation at 10 Hz for a period of 15 s.*
It also shows the effect of naloxone on the inhibitory response.

There is a significant difference between the IR obtained in control conditions and the presence of naloxone either in ileum ($p < 0.05$) or duodenum ($p < 0.001$). The decrease of IR in the presence of naloxone is significantly higher in duodenum ($p < 0.05$). In addition, we have studied the effect of methionine-ekephalin (Met-E) on the MPLM ileum and duodenum. The inhibitory response obtained in both intestinal segments was similar; the CI_{50} obtained were 1.5 ± 0.2 ($\times 10^{-7}$M) $n = 6$ and 1.03 ± 0.05 ($\times 10^{-7}$M) $n = 6$ respectively.

The results clearly show that there is a significantly higher IR after stimulation at 10 Hz in duodenum than in ileum. We believe that this increase of the IR in duodenum is produced by an increased of the endogenous opioid release and is not due to a change in the affinity receptors since the MPLM strips obtained from duodenum have the same sensitivity to Met-enkephalin as the strips obtained from ileum.

The greater effect of naloxone as antagonist in duodenum can be explained by a higher release of endogenous opioid in this region of the intestinal tract.

Key words: Opioid peptides release, Electrical stimulation, Myenteric plexus.

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