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INHIBITION OF ENKEPHALIN DEGRADATION IN THE GUINEA PIG ILEUM

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Summary

Guinea pig ileum tissue preparations contain enzymes which degrade both leucine and methionine enkephalin by cleavage of the N-terminal tyrosine residue. Similar enkephalin degrading activity is also found in the fluid bath surrounding ileum tissue preparations and appears to arise from serum and broken cell enzymes. Chelating agents such as 1,10-phenanthroline and 8-OH quinoline are effective inhibitors of enkephalin destruction by these enzymes but in the concentrations necessary to inhibit all enzyme activity, they disturb the contractility of the ileum during in vitro bioassays. The presence of enkephalin degrading enzymes and the lack of appropriate peptidase inhibitors may hinder the determination and quantification of enkephalin release in this tissue.

We have previously demonstrated that electrical stimulation of the guinea pig ileum myenteric plexus-longitudinal muscle (MPLM) tissue preparation releases endogenous substances which activate opiate receptors (1,2) and that there is cross tolerance between the electrically induced release of endorphins and morphine (2,3). Although the nature of the substances released by electrical stimulation has not yet been determined, it is likely that they could be enkephalins. The myenteric plexus is known to contain enkephalin (4) and both leucine and methionine enkephalin have been reported to be released from MPLM strips by electrical stimulation at 0.1 Hz (5). Smith et al. (6) have demonstrated that potassium releases enkephalin from guinea pig ileum segments by a calcium dependent mechanism.

To date our attempts to identify enkephalin in the tissue bath after stimulation at 10 Hz have been unsuccessful. Therefore, we feel that if enkephalin is being released, most of it may be destroyed by tissue peptidases before we can detect it (7,8). The presence of enzymes in the guinea pig ileum that degrade enkephalin has previously been described (9-11). However, the nature of these enzymes has not been studied.

The present work was undertaken in order to partially characterize some of the peptidases responsible for enkephalin degradation in guinea pig ileum preparations. Emphasis was placed upon searching for suitable enzyme inhibitors which could be used for the study of enkephalin synthesis and release in this tissue preparation.

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Materials and Methods

Methionine and leucine enkephalin were purchased from Beckman Bioproducts and Calbiochem. (Tyrosyl-3,5-^{3}H)-methionine enkephalin (16.1 Ci/mmole) and (tyrosyl-3,5-^{3}H)-leucine enkephalin (45.6 Ci/mmole) were obtained from New England Nuclear. Trasylol^R was obtained from FBA Pharmaceuticals. Amberlite XAD-2 polystyrene resin (20-50 mesh) was purchased from Mallinkrodt. All other compounds were procured from commercial sources.

Preparation of MPLM Strips

White English short hair male guinea pigs (300-400 g) were used throughout this study. Animals were killed by a blow on the head. The ileums were removed and washed with Krebs-Ringer bicarbonate buffer gassed with 95% O_2-5% CO_2 and maintained at 37°C. MPLM strips (20-30 mg tissue per strip) were prepared as described by Kosterlitz et al. (12) and kept in gassed Krebs bicarbonate buffer at 37°C.

Preparation of Intact Ileum Segments

A washed ileum was cut into 5 cm segments. Each segment was invaginated at both ends and tied closed so that only the serosal side was in contact with the surrounding Krebs medium. The ileum segments were kept in Krebs buffer at 37°C and gassed with 95% O_2-5% CO_2.

Preparation of High Speed Supernatant Enzymes

Guinea pig brain and MPLM strips were homogenized in 10 volumes of ice-cold isotonic sucrose using a Polytron tissue homogenizer. The homogenates were centrifuged at 100,000 x g for 1 hour and the resulting supernatant fractions were stored at -20°C.

Determination of Enkephalin Degradation

Enkephalin degradation was studied in MPLM strips, intact ileum segments, serum, 100,000 x g supernatant fractions of MPLM strips and brain, and also in the Krebs medium surrounding MPLM strips and intact ileum segments.

Degradation of enkephalin by MPLM strips or intact ileum segments was studied by incubating each tissue preparation with 2×10^{-8} M (tyrosyl-3,5-^{3}H)-methionine enkephalin or (tyrosyl-3,5-^{3}H)-leucine enkephalin in 2 ml Krebs bicarbonate buffer at 37°C, gassed with 95% O_2-5% CO_2; blank samples did not contain tissue. At the end of the incubation, the tissues were removed and HCl added to a final concentration of 0.2 N; the HCl contained cold enkephalin and tyrosine to act as carriers. Aliquots of the incubation medium were spotted onto silica gel plates which were routinely developed in butanol: acetic acid:ethyl acetate:water (1:1:2:1); this system effectively separates methionine and leucine enkephalin from all tyrosine-containing degradation products (methionine enkephalin, R_f=.77; leucine enkephalin, R_f=.81; tyrosyl-glycyl-glycyl-phenylalanine, R_f=.61; tyrosyl-glycyl-glycine, R_f=.39; tyrosyl-glycine, R_f=.47 and tyrosine, R_f=.55). Alternate solvents used included chloroform:methanol:ammonium hydroxide (60:30:5) and ethanol:water (70:30). The developed plates were sprayed with ninhydrin and the spots corresponding to enkephalin and tyrosine were scraped into scintillation vials. Tyrosine and enkephalin were eluted off the silica with 1 ml methanol; 10 ml of scintillation fluor were then added to each vial and the amount of radioactivity determined by liquid scintillation spectrometry at a counting efficiency of 40%.

When compounds were screened for their ability to inhibit enkephalin degradation by MPLM strips, an alternate procedure was used. Incubations