Enkephalin release from the myenteric plexus of the guinea-pig small intestine in the presence of cycloheximide

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The myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum has been shown to be a useful model for the study of the biogenesis of methionine- and leucine-enkephalin (Sosa, McKnight, Hughes & Kosterlitz, 1977). Since the incorporation of [^{3}H]-tyrosine is suppressed by puromycin or cycloheximide, a measure of the release may be obtained by determining the enkephalin content of the tissue before and after electrical field stimulation in the presence of these drugs.

Preparations (about 300–400 mg) from the small intestine of one guinea-pig were suspended at 37°C in organ baths (3 ml) containing Krebs solution to which had been added ascorbic acid (20 μg/ml) and the following amino acids (1 μg/ml): Ala, Arg, Asp, Cys, Glu, Gly, His, Leu, Ileu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val. The solution was bubbled with 95% O_2 and 5% CO_2. After 30 min pre-incubation, cycloheximide was added to give a concentration of 0.1 mM and the incubation continued for a further 30 min, when electrical field stimulation (0.5 ms, supramaximal voltage) was started for periods of 0.5 to 4 hours. At the end of stimulation, the total enkephalin content, expressed as methionine-enkephalin, was determined after extracting the tissues with 0.1 M HCl, purification by adsorption on XAD-2, elution with methanol and assay on the mouse vas deferens (Hughes, Kosterlitz & Smith, 1977).

Stimulation at 10 Hz reduced the enkephalin content by 16% ($n = 2$) in 30 min and 46 ± 5% ($n = 4$) in 60 minutes. There was no further significant reduction after stimulation for 120 min (43 ± 6%; $n = 4$). At a frequency of 1 Hz, the loss of enkephalin was about 10% of that observed at 10 Hz; no loss could be detected after stimulation at 0.1 Hz for 4 hours. The reduction in the enkephalin content due to stimulation was abolished by tetrodotoxin (0.3 μM).

From the values obtained, the fractional release per pulse has been estimated to be about 10^{-5} for stimulation frequencies of 1 and 10 Hz. It follows that the amount released per pulse is of the order of 0.003 ng/g of tissue for an average tissue content of 285 n/g. These estimates may be too low because it has not been finally established that 0.1 mM cycloheximide blocks synthesis completely and because nothing is known about the size of the precursor pools present at the beginning of electrical stimulation. It would therefore be premature to compare our results with those of Schulz, Wüster, Simantov, Snyder & Herz (1977) who, after stimulation at 0.1 Hz, found a large release of enkephalin-like material into the bath fluid (0.14–7 ng/g of tissue per pulse).

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