The incorporation of [^{3}H]-tyrosine into the enkephalins of striatal slices of guinea-pig brain

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We have recently shown that the longitudinal muscle-myenteric plexus preparation of the guinea-pig ileum is able to synthesize the two opioid pentapeptides methionine-enkephalin and leucine-emkephalin and that this synthesis is inhibited by cycloheximide and puromycin (Sosa, McKnight, Hughes & Kosterlitz, 1977). The present experiments were to determine whether it could be shown that a similar synthesis occurred *in vitro* in striatum from guinea-pig brain.

Individual striata from male guinea-pigs weighing 300–500 g were cut, approximately in the sagittal plane, on a McIlwain tissue chopper into slices of 0.5 mm thickness. The slices were incubated at 36°C for 30 min in 10 ml Krebs solution containing 22 mM glucose and 50 μCi L-[2,3,5,6-^{3}H]-tyrosine (80 Ci/mmol, Radiochemical Centre Amersham). The rate of flow of a mixture of 95% O_2 and 5% CO_2 through this solution was just sufficient to prevent settling and clumping of the slices. After this initial labelling period, the bathing solution was changed to 10 ml Krebs solution containing the following amino acids (1 μg/ml): Ala, Arg, Asp, Cys, Glu, Gly, His, Ileu, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val and incubation continued for up to 7 hours.

After incubation, the incubation fluid was replaced with ice-cold 0.1 M HCl and the slices were filtered off and homogenized in 15–20 ml of 0.1 M HCl containing methionine-enkephalin (40 μg) and leucine-enkephalin (20 μg). The homogenates were centrifuged at 50,000 *g* for 20 min and the enkephalins isolated by the following chromatographic steps: adsorption onto XAD-2, cation exchange on HC-Pellionex-SCX, desalting by XAD-2 adsorption and anion exchange on AE-Pellionex-SAX (see Sosa *et al.*, 1977). In the final thin layer chromatographic stage, the silica gel plate was developed for 11–12 cm with ethyl acetate/pyridine/water/acetic acid (100:43:25:11) containing 0.01% (v/v) ethane-1,2-dithiol to minimize oxidation of the S of methionine-enkephalin to the sulphoxide. Bands 7–8 mm wide were eluted in 1 ml 80% (v/v) methanol and the radioactivity found in the bands corresponding to methionine- and leucine-enkephalin was taken as an index of their synthesis.

The incorporation of [^{3}H]-tyrosine into the enkephalins showed three distinct phases. There was an initial lag phase lasting 2 h, followed by a linear phase of increasing incorporation over the next 3 h reaching a maximum of 962 ± 37 $dmin^{-1}g^{-1}$ wet tissue and $501 \pm 20 dmin^{-1}g^{-1}$ for methionine- and leucine-enkephalin ($n = 8$), respectively. The amount of incorporated [^{3}H]-tyrosine remained fairly constant during 6 to 7 h after labelling. The incorporation of [^{3}H]-tyrosine into the proteins of the guinea-pig striatum was also measured after precipitation of the proteins of the 50,000 *g* pellet by 5% (w/v) trichloroacetic acid. After centrifugation the proteins were dissolved in 1 M NaOH and an aliquot was taken for scintillation counting. The rate of incorporation of [^{3}H]-tyrosine into the proteins continued linearly for up to 7 hours.

We conclude that the enkephalins of the guinea-pig striatum can be synthesized locally, possibly from a precursor or precursors also produced locally and that this preparation appears to be a useful model for the *in vitro* investigation of the synthesis of enkephalins.

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Reference

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