OPIOID RADIORECEPTOR ASSAY, MET^5-ENKEPHALIN RADIOIMMUNOASSAY AND β-ENDORPHIN RADIOIMMUNOASSAY

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Eendorphins, isolated from brain and pituitary, bind opiate receptor, but the physiological significance of this peptide remeins to be defined. Three sensitive and specific assay systems for endorphins have been developed. (1) Opioid radioreceptor assay (RRA) : [^{3}H]-naloxone, a potent opiate antagonist, was incubated with rat brain (minus cerebellum) homogenate in ice for 2 hr. Met^5-enkephalin was used as a standard in RRA. (2) Met^5-enkephalin (M-ENK) radioimmunoassay (RIA) : M-ENK was conjugated with bovine serum albumin using glutaraldehyde. This complex emulsified with Freund's complete adjuvant was injected into rabbits to generate antibodies. M-ENK was iodinated with ^{125}I using lactoperoxidase and H_2O_2, and purified on a carboxymethyl-cellulose column using ammonium acetate buffers. Antisera to M-ENK (3 of 4 rabbits) at a dilution 1:4,000 bound 45% of ^{125}I-M-ENK. This antiserum has no cross-reactivity with α-endorphin, β-endorphin, somatostatin, TRH, LH-RH and MSH-RIH, and has a small degree of cross-reactivity with Leu^5-enkephalin. (3) β-endorphin (β-E) RIA : Antiserum to β-E was made using simillar method to generating antisera to M-ENK. β-E also iodinated with lactoperoxydase method. Antiserum to β-E bound 55% of ^{125}I-β-E at a final dilution of 1:60,000. The cross-reactivity of this antiserum is <0.02% with M-ENK, Leu^5-ENK and α-endorphin. Using these three assay systems, total enkephalins activity and immunoreactive M-ENK found in rat brain have the regional distribution correlating with that of opioid receptors. However, β-E-like immunoreactivity has a different regional distribution from that of opioid receptors and that of enkephalins.

ON THE DEGRADATION OF β-ENDORPHIN BY RAT BRAIN CYTOSOL

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Among opiate-like peptide (endorphins) from brain and pituitary extracts, β-endorphin has the most potent analgesic activity. β-Endorphin was shown to be identical to amino acid seqences 61-91 of β-lipotropin. We studied degradation of β-endorphin incubated with rat brain cytosol and the effect of various drugs on the degradation of β-endorphin.
Wister rats were killed by decapitation and the brain tissue were homogenized in 30 volume of ice-cold physiological saline with a Brinkman Polytron PT-10 apparatus. the homogenates were centrifuged at 100,000×g for 60 min. and supernatants (cytosol fraction) were used as adegradating enzyme. In a routine experiment the incubation of β-endorphin was carried out in a mixture consisting of 15mg of rat brain cytosol with or without bacitracin (50ug/ml), Trasylol (2000 KIU/ml) or phenylmethyl sulfonyl fluoride (PMSF 2mM) for 10-60 min. at 37°C. The reaction was terminated by adding acetic acid and then immersing in boiling water for 5min. The reaction mixtures were then centrifuged at 2,000×g for 30min., and the supernatants were assayed by β-endorphin radioimmunoassay. β-Endorphin was degraded rapidly by cytosol of rat brain and half-life of β-endorphin in this experimental condition was 5.5min. Bacitracin and Trasylol considerably reduced degradation of β-endorphin inactibated with rat brain cytosol.