RADIOIMMUNOASSAY FOR β-ENDORPHIN

Kazuwa Nakao, Shogo Oki, Yoshikatsu Nakai and Hiroo Imura

Second Division, Department of Medicine, Kyoto University, School of Medicine, Kyoto.

A sensitive radioimmunoassay for β-endorphin has been developed. The antiserum to β-endorphin was prepared in a guinea pig by several bi-weekly injections of 10 U of crude porcine ACTH-Z (Organon) emulsified in complete Freund's adjuvant (Difco). This was used at a titer of 1:600,000. Synthetic human β-endorphin (C. H. Li) was labelled with $Na^{125}I$ using chloramine T according to the method of Hunter and Greenwood. Purification of ^{125}I-β-endorphin was performed by absorbing to Quso G-32. The specific activity of ^{125}I-β-endorphin ranged from 100 to 200 μCi/μg. Radioimmunoassay for β-endorphin was performed by talc absorption method, previously described in detail for the radioimmunoassay of ACTH (Horm. Metab. Res. (Suppl)5:7, 1975). Labelled β-endorphin and standard β-endorphin (C. H. Li) or unknown samples were incubated with the antiserum for 3 days at 4°C. The separation of antibody-bound from free labelled hormone was done by absorption of free fraction to 50 mg of talc. The minimal detectable quantity of β-endorphin was 1 pg. Human β-endorphin and human β-lipotropin (C. H. Li) equally displaced ^{125}I-β-endorphin from the antiserum, when compared on a molar basis, but human ACTH, α-MSH, human β-MSH, α-endorphin, γ-endorphin, Leu^5-enkephalin and Met^5-enkephalin failed to displace ^{125}I-β-endorphin from the antiserum, even when quantities as much as 10 ng were added. Due to the cross reactivity of this radioimmunoassay with β-lipotropin, β-endorphin levels were evaluated by gel exclusion chromatography. Two peaks with β-endorphin immunoreactivity were found in plasma extract from a patient with Nelson's syndrome; one peak eluted in the position compatible with ^{125}I-βh-lipotropin and another in the position of ^{125}I-βh-endorphin. The dilution curve of fraction compatible with the molecular weight of β-endorphin was observed to be parallel to the standard curve of β-endorphin. Using this radioimmunoassay and gel exclusion chromatography, we have demonstrated the existence of β-endorphin in plasma from normal subjects and a patient with Addison's disease, in human cerebrospinal fluid, in human placenta and in media of AtT-20 cell line or ACTH producing human carcinoid cell line.

A RADIOIMMUNOASSAY FOR SUBSTANCE P

Yasuhide Yamawaki, Norio Ogawa, Jiro Takahara and Tadashi Ofuji

Third Department of Internal Medicine, Okayama University Medical School, Okayama.

Substance P (SP) is a potent hypotensive peptide first detected in extracts of equine brain and intestinal tissue. A sensitive and specific radioimmunoassay (RIA) for SP have been developed. Synthetic SP (Peptide Institute, Osaka) was conjugated with bovine serum albumin using glutaraldehyde. This complex was emulsified with Freund's complete adjuvant and injected into rabbits to generate antibodies. Nα-Tyr-SP (supplied by Dr. N. Yanaihara) was iodinated with ^{125}I using lactoperoxidase and H_2O_2, and purified on Sephadex G-10 colmn using 0.3 M acetic acid-6 M urea. Two radioactive peaks were eluted, the descending part of the peak exhibiting greatest binding to antiserum. Antisera to SP bound 50% of ^{125}I-Nα-Tyr-SP at a final concentration of 1:250,000 by dextran-coated charcoal method. In the RIA, assay buffer was added 5 mM of mercaptoethanol and assay tubes were incubated at 4°C for 18-24 hr. Sensitivity was usually 3 pg/tube. TRH, LH-RH, somatostatin, MSH-RIH, VIP, neurotensin and β-endorphin had no cross-reactivity in this RIA. Using synthetic SP fragments (supplied by Dr. N. Yanaihara), this antiserum was shown to react to amino acid sequences 6-11 of SP.

Immunoreactive SP in extracts of male rat forebrain, midbrain plus hindbrain and pituitary gland were 17 ng/g, 260 ng/g and 130 ng/g, respectively.