Recent advance in Radioassay

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RECENT ADVANCE IN RADIOIMMUNOASSAY FOR LOW MOLECULE PEPTIDES. H. Saito The University of Tokushima School of Medicine, Tokushima

For accurate estimation of the concentrations of low molecule peptides in the biological materials by radioimmunoassay(RIA), it is necessary to investigate 1) the region of antigenic determinant of the prepared antibody, 2) the mode of existence and characteristics of the immunoreactive substances in samples and 3) the evaluation of errors in RIA system due to non-specific interferrence and the countermeasures to aboid them.

Recently, we established sensitive and specific RIAs for measuring somatostatin, atrial natriuretic polypeptide, buserelin(an analog of LH-RH) and growth hormone-releasing hormone in the plasma and obtained the following findings.

Somatostatin(SS): In normal subjects the fasting plasma SS concentration were 13.3 ± 5.3 pg/ml(mean ± SD). Very high values of plasma SS, ranging from 125.0 - 400.0 pg/ml, were found in all four patients with medullary carcinoma of the thyroid examined and such SS levels were changed in parallel with their clinical course after resection of the tumor. In addition, in the preoperative state of medullary thyroid carcinoma, an exaggerated response in SS release was observed after the intravenous arginine infusion, suggesting that SS was released from the tumor.

Atrial natri-uretic polypeptide(ANP):ANP is a novel peptide hormone which is thought to act directly on the kidney via general circulation. However, the basal plasma level of ANP in normal subjects was considerably low(19.3 ± 1.0 pg/ml; applox. 5×10^{-12}M) and the peptide was easy to be inactivated by protease in the plasma(stability at 37°C for 30 min was less than 50 %). From this, it was known that blood samples should be collected in the chilled test tubes containing protease inhibitor such as aprotinin to prevent the peptide from degradation.

Buserelin:Recently, buserelin(a super agonist of LH-RH) has been given subcutaneously or intranasally for treatment of various diseases such as prostate cancer and endometriosis. Therefore, to clarify the pharmacokinetics and bioavailability of this compound, we set up RIA for buserelin. The half disappearance time of buserelin(118 ± 26 min)was much longer than that of authentic LH-RH(3-6 min). There was no difference in the area under the curve for plasma levels between the groups of subcutaneous injection of 5 μg and intranasal administratation of 450 μg.

Growth hormone-releasing factor(GRF): The fasting plasma levels of GRF in normal adults were 10.3 ± 4.1 pg/ml (mean ± SD) and there was no sex difference. A marked episodic GRF release could be detected in the peripheral circulation just before the GH surge that is observed in the initial slow wave stage of sleep at night. In 18 out of 23 patients with idiopathic pituitary dwarfism studied, the GRF was undetectable in plasma(<4 pg/ml), suggesting that primary lesion was at the level of the hypothalamus, not the pituitary.

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BACKGROUND AND APPLICATION OF RADIORECEPTOR ASSAY (RRA). T.Tsushima, Y.Sato and N. Emoto. Department of Medicine,Tokyo Women's Medical College.

RRA is a competitive radioassay based on interaction of hormone with its receptor. The activity of hormones determined by RRA agree well with the biological activity.RRA has been applied to studies on structure-function relationship, and to detection of hormone-like substances or antibody (Ab) to hormone-receptors. Sensitivity of RRA is less than that of RIA, but this problem can be overcome by modulation assay utilizing down-regulation of receptors. Anti-insulin receptor Ab in sera of patients with type B insulin resistance can be detected by their ability to inhibit I-125 insulin binding to receptor or to immunoprecipitate I-125 crosslinked solubilized receptor. Immunoprecipitation (IP) method was quite sensitive to detect Ab to insulin receptor. IgG from patients with this syndrome was subjected to isoelectric focusing, and 30 fractions each were assayed by insulin-RRA, IP method and insulin biossay (BA). The distribution of these 3 activities was not always identical. Some fractions had IgG with both RRA- and IP-activity, but some had IgG with RRA-activity alone. The latter type of IgG was found to have an inhibitory effect on insulin-induced lipogenesis in rat adipocytes, while IgGs with the two activities consistently showed insulin-like activity. Thus, Ab to insulin receptor was heterogenous.