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A MEASUREMENT OF METANEPHRINE AND NORMETANEPHRINE BY RADIOIMMUNOASSAY. H.Tashiro, A.Takeyasu and T.Ishigami. Kitasato Biochemical Laboratories (Bristol-Myers KK) Sagamihara.

A measurement of metanephrine and normetanephrine concentrations is valuable to diagnose pheochromocytoma and neuroblastoma. Metanephrine and normetanephrine are intermediate metabolites of catecholamines. Fluorometry,gas chromatography mass spectrometry and high-performance liquid chromatography(HPLC) have been used to measure metanephrine and normetanephrine. But,these methods have some problems,which are at extraction and purification steps. They are very tedious and time-consuming. We have recently studied a measurement of metanephrine and normetanephrine using radioimmunoassay(RIA).These assay kits uses a one step and double-antibody method,and I-125 labeled synephrine and octopamine as a tracer. Metanephrine and normetanephrine in plasma,serum and urine could be measured directly without extraction and purification. Intra-assay coefficients of variation was below 10%,and the results of dilution and recovery tests were good. The sensitivity of the assay was satisfactory in plasma and serum from normal group. The correlation between RIA and HPLC that we have used was good. Results of our studies suggest that these RIA kits would be available of simple and direct measurements of metanephrine and normetanephrine in plasma serum and urine.

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RADIOIMMUNOASSAY of CGRP.K.Kanao, S.Ishihara, M.Honda, M.Usami, H.Ochi and H.Morii. Sumitomo Hospital and Osaka City University.,Osaka.

Calcitonin Gene Rerated Peptide(CGRP)is a newly discovered peptide of 37 amino acids, and has been identified in central and peripheral neural tissues and others. We reported fundamental evaluation on CGRP radioimmunoassay. The CGRP assay was conducted by adding to 100 µl of either standard(0-5,000 pg/ml) or sample, 100 µl of 150tube/vial diluted rabbit antiserum against hCGRP(Amersham), 500 µl of 50mM sodium phosphate buffer at pH7.4 containing 0.3%BSA, 10 mM EDTA, 0.1% NaN3.

The mixtures was incubated overnight at 4°C, and then 100 µl of I-125 hCGRP(less than 10^{-8}fmol/tube,Amersham) were added in each test tubes. The mixtures were incubated at 4°C for three days. Antibody bound CGRP was then precipitated by adding of 100 µl of second antibody. Sensitivity was 40 pg/ml and normal value(20-40 years) was 80-280 pg/ml(n=15). There was no interference with the assay from Calcitonin, Osteocalcin and Somatomedin-C.

Serum CGRP in three patients with MTC (Medullary thyroid carcinoma) was 260,570 and 15,000 pg/ml respectively. Gel permeation chromatography of this MTC sample, shows 15,000 pg/ml, extractable CGRP-like immunoreactivity revealed two distinct immunoreactive peaks.

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RADIORECEPTOR BINDING OF CHOLECYSTOKININ TO GASTRIC CHIEF CELLS. M.Noguchi,H.Adachi, S.Sato,T.Honda,S.Ohnishi,E.Aoki and K.Torizuka*. Kyoto University School of Medicine, Kyoto and Fukui Medical School*, Fukui.

Cholecystokinin (CCK) stimulates pepsinogen secretion from gastric chief cells of guinea pig. We examined radioreceptor binding of CCK to isolated chief cells from guinea pig stomach. Gastric chief cells were prepared from gastric mucosa by enzyme digestion using collagenase and subsquent centrifugation with percoll density gradient. Natural CCK-33 was iodinated by the method of Bolton-Hunter. Binding of I-125 labeled CCK to chief cells was saturable, reversible and dependent on temparature. Unlabeled CCK, but not VIP, inhibited the binding. Hofstee plot (modified Scatchard plot) obtained from dose-response inhibition curve of CCK-8 for the binding, showed that CCK receptor on chief cell possessed one high-affinity binding site (Kd value; 0.4 nM). When compared the action of CCK for the binding with that for pepsinogen secretion from chief cells, both response-curves consisted with each other, indicating that the receptor binding of CCK to chief cells is physiological. In addition, IgG purified from control sera using Protein A affinity column, failed to affect the binding at the concentration below 50 µg/ml. These results suggest that the receptor binding assay of CCK to chief cells is useful to detect anti-chief cell antibody in serum of patients with pernicious anemia.

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RADIOIMMUNOASSAY FOR 11-DEOXYCORTICOSTERONE USING NEW HAPTEN. M.Hachinoe,T.Tanaka, H.Harada and A.Kubodera. Faculty of Pharmaceutical Sciences, Science University of Tokyo, Tokyo.

Several types of haptens have been made to prepare antisera for the radioimmunoassay (RIA) of 11-deoxycorticosterone (DOC). The antisera so far obtained, however, are not yet satisfactory with the specificity. Therefore we prepared the DOC-BSA conjugate having the bridge at the C-4 position in the Δ^4-3-ketosteroid molecule which was used to yield the specific antisera for DOC, and attempted to establish the RIA for DOC.

DOC was transformed into the 4-(2-carboxyethylthio) ether as a hapten, and the male guinea pigs were immunized with the hapten-BSA conjugate. The affinity, sensitivity and specificity of the resulting antisera were assessed.

The antisera exhibited the high affinity for DOC with an affinity constant (Ka) of 1.27×10^9 M^{-1}. The RIA method is capable of determing DOC in the range of 20-1000 pg by using H-3-DOC (s.a. 42 Ci/mmol). The antisera could well recognize the ring A and the functional groups on the C-17 side chains, and the specificity of them was improved as compared with the conventional antibodies. Furthermore, the antisera were purified by eliminating cross-reactive antibodies by affinity chromatography, and the specificity of the purified antisera was assessed.