Presented by Medical*Online


A measurement of metanephrine and normetanephrine have been identified in central and peripheral neural tissues and others. We report fundamental evaluation on CCRP radioimmunoassay. The CCRP 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 antisera against hCCRP(Amersham), 500 μl of 25M sodium phosphate buffer at pH7.4 containing 0.2%BSA, 10 mM EDTA, 0.1% NaN3.

The mixtures were incubated overnight at 4°C, and then 100 μl of 1-125 hCCRP(less than 10−6mol/tube, Amersham) were added in each test tubes. The mixtures were incubated at 4°C for three days. Antibody bound CCRP was then precipitated by adding of 500 μl of secondary 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 CCRP 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 CCRP-like immunoreactivity revealed two distinct immunoreactive peaks.

RADIOIMMUNOASSAY to GASTRIC CHIEF CELLS. M.Noguchi, M.Adachi, S.Sato, T.Honda, S.Ohishi, 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 radioimmunoassay binding of CCK to cells from guinea pig stomach. Gastric chief cells were prepared from gastric mucosa by enzyme digestion using collagenase and subsequent 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 temperature. Unlabeled CCK, but not VIP, inhibited the binding. Scatchard 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 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 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 peptic ulcer.

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

Several types of haptons 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 43-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 (Ko of 1.27 x 109 M−1). The RIA method is capable of determining DOC in the range of 20-1000 pg by using H-3-DOC (a.a. 42 Cl/mmol). The antisera could well recognize the 11-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.