

Determination of Human Plasma Oxytocin by Radioimmunoassay

S. OGAWA, S. FUKUCHI, T. MIURA, K. NAKAJIMA

Third Department of Internal Medicine

H. TAIRA, M. SAITO

Laboratory of Radioisotope, Fukushima Medical College, Fukushima

To produce antibodies to oxytocin, an antigen was prepared according to the following procedure: Synthetic oxytocin, 10 mg, and porcine gamma globulin, 10 mg, were dissolved in 4 ml of distilled water and the pH was adjusted to 6.3, and then 150 mg of carbodiimide was added. The solution was incubated at 25°C for 4 hours and dialyzed overnight at 4°C against distilled water. This antigen was mixed with an equal volume of Freund's complete adjuvant and injected intramuscularly to rabbits at 2 weeks intervals.

Radioiodination of oxytocin was performed by chloramine-T method and ^{125}I oxytocin was separated by gel filtration using Sephadex G-25.

The antibody showed a slight cross-reactivity with both lysine-8-vasopressin and arginine-8-vasopressin, but did not significantly with angiotensin I, II and ACTH.

On an assay, all dilutions were made with 0.05 M phosphate-saline buffer, pH 7.4, containing 0.1% lysozyme. Samples were diluted and added with ^{125}I oxytocin and then with 0.1 ml of rabbit anti-oxytocin serum diluted 1/500. The mixture was incubated at 4°C for 72 hours and the antibody-bound antigen was separated using dextran-coated charcoal. The sensitivity of this assay was 4

pg/tube.

To determine plasma oxytocin concentration, plasma samples were adsorbed by florisil and extracted with acetone containing 1% acetic acid. The adsorption and extraction capacities were 96.6 ± 1.5 and $79.1 \pm 1.9\%$ respectively.

The mean plasma concentrations of oxytocin in healthy males and females were 0.66 ± 0.95 ($n=9$) and 8.6 ± 1.13 ($n=2$) pg/ml respectively.

Those in the first and the third stage of labor and in the puerperium (2nd to 8th day) were respectively 19.7 ± 7.8 ($n=7$), 22.3 ± 11.2 ($n=7$) and 8.2 ± 6.0 ($n=6$) pg/ml.

In the females undergoing labor, compared with the healthy males and non-pregnant females, a concentration of oxytocin rose significantly. Each aliquot of the samples was frozen for storage and the assay was repeated weekly for a total of three weeks. The concentrations of oxytocin reduced gradually to 63.7, 43.0 and 26.3% of the initial values with increasing weeks.

From these results the radioimmunoassay for oxytocin is considered to be capable of being clinically applied, although there remained some problems concerning the preservation of samples and the specificity of the assay.