SHORT COMMUNICATION

THE INTERFERENCE OF HUMAN IgG WITH THE DOUBLE-ANTIBODY RADIOIMMUNOASSAY OF THYROTROPHIC HORMONE AND ITS CLINICAL SIGNIFICANCE

C. CHAPMAN, W. N. HUTTON* AND C. J. HAYTER

Department of Nuclear Medicine, The General Infirmary at Leeds, and
*Otley General Hospital, Otley

(Received 17 January 1974)

SUMMARY

1. High values for plasma thyrotrophic hormone, which did not correlate with other parameters of thyroid function, were found in patients with abnormally high plasma immunoglobulin concentrations.

2. Results indicate that human IgG interferes with the post-precipitation double-antibody radioimmunoassay of thyrotrophic hormone. It is possible that this interference occurs with similar radioimmunoassays of other substances.

Key words: thyrotrophic hormone, double antibody radioimmunoassay, paraproteinaemia, immunoglobulin G.

There are many unsolved problems associated with radioimmunoassay and it has been recognized that some of these are caused by the interference of unidentified plasma components (Morgan, Sorenson & Lazarow, 1964; Burr, Grant, Sizennko, Kaplan & Grumbach, 1969). During investigations in this field, it was noted that a plasma sample apparently had a high concentration of thyrotrophic hormone (TSH), which did not correlate with the results of other thyroid function tests. The patient from whom this sample was obtained had IgG paraproteinaemia and a study of patients with paraproteinaemias was therefore undertaken.

METHODS

Plasma thyroxine was measured with a Thyopac-4 kit (The Radiochemical Centre, Amersham, Bucks). Thyroid hormone-binding proteins were assayed by measuring competition between them and charcoal for tracer quantities of $^{125}$I-labelled tri-iodothyronine; the method was

Correspondence: Dr C. Chapman, Department of Nuclear Medicine, The General Infirmary at Leeds, Great George Street, Leeds LS1 3EX.

651
similar to that of Herbert, Gottlieb, Lau, Gilbert & Silver (1965). Results obtained with patients’ sera were expressed by relating the results to a standard serum, which had arbitrarily been assigned a value of 1.0. A free thyroxine index was obtained by multiplying the result obtained from the charcoal-binding procedure by the result obtained from the Thyopac-4 measurement.

TSH was measured by a modification of the method of Odell, Wilber & Paul (1965), in which 0.2 ml plasma samples were assayed (instead of 0.3 ml) and the amounts of the other constituents (human chorionic gonadotrophin, ethylenediaminetetra-acetate, first antibody and 125I-labelled TSH) were decreased correspondingly. We have shown that when the constituent reaction solutions are pooled 1–2 h before addition to plasma samples, the same results are obtained as when these solutions are added individually. There is considerable saving in work and we have, accordingly, adopted this modification. The samples for standard curves were made up in buffer (Odell et al., 1965) containing human serum albumin (1 g/l). Plasma was not added to the standards. Rabbit anti-(human TSH) antibody was supplied by the National Pituitary Agency and the standard human TSH was batch 68/38 supplied by the Medical Research Council. Iodination of TSH was by the method of Greenwood, Hunter & Glover (1963). The second antibody, which was added 5 days after the start of the reaction, was from sheep immunized with rabbit γ-globulin Cohn fraction II (Koch–Light Laboratories Ltd).

Total protein and albumin concentrations in serum were measured in an Autoanalyser mark II (Technicon Instruments Company Ltd, Basingstoke, Hants). The method for total protein was as described in the Technicon method sheet AAII-14; the method for albumin was that described by Northam & Widdowson (1967), modified for the Autoanalyser mark II. Immunoglobulins IgA, IgG and IgM were measured with radial immunodiffusion kits (Dade Division, Hospital Supply Corporation, Miami, Florida).

The patients studied were attending a routine haematology clinic and all had paraproteinaemia (Table 1).

RESULTS AND DISCUSSION

The results are shown in Table 1. The linear correlation coefficients of TSH with the other parameters were (n.s. = not significant): T₄, −0.17 (n.s.); free T₄ index, −0.05 (n.s.); total protein, 0.67 (P < 0.01); albumin, 0.00 (n.s.); IgA, −0.39 (n.s.); IgG, 0.95 (P < 0.001); IgM, −0.34 (n.s.).

Normally the amounts of TSH found in the samples containing high concentrations of IgG (Table 1) would indicate a diagnosis of primary hypothyroidism. However, there was no clinical evidence in any of the patients to suggest abnormal thyroid function. In general, this clinical opinion was confirmed by the other data relating to thyroid function. Although three of the patients had borderline low results for plasma total thyroxine, two of them had normal values for free thyroxine index. Only one patient (number 9, Table 1) had low results for both total thyroxine and free thyroxine index. There was no evidence then or later for primary hypothyroidism but it is possible that in this case the high TSH value was genuine.

The very significant correlation between plasma TSH and IgG concentrations can be explained by the nature of the post-precipitation double-antibody reaction (Midgley, Rebar & Niswender, 1969). Basically, two reactions are involved. The first, TSH + Ab₁ ⇄ TSH − Ab₁, is concerned with the competition between radioactive TSH and stable TSH for the binding sites on the first antibody (Ab₁); the amount of radioactive TSH bound to this antibody is inversely
Our results indicate that human IgG interferes with the double-antibody assay of TSH. It is tempting to suggest that this is caused by human IgG competing with the first antibody (containing rabbit IgG) for the second antibody, i.e. that sheep anti-(rabbit γ-globulin) cross-reacts with human IgG. If this were so, human IgG would decrease the amount of radioactive TSH precipitated, thus giving rise to anomalously high results.

If it is true that IgG influences the TSH assay, it is likely that it will also influence results of other post-precipitation double-antibody assays. This is likely to be of importance because many disease states are associated with elevated IgG concentrations (Hobbs, 1970). The TSH assay is particularly vulnerable in this respect because of the high incidence of autoimmunity, associated with abnormal concentrations of plasma IgG in thyroid disease.

Although we obtained erroneously high assay values of plasma TSH in patients with paraproteinaemia, this need not always be the case. For example, if the standards used in an assay included plasma which happened to contain considerable quantities of IgG, then the unknown plasmas would all read low. This sort of error might account for some of the discrepancies between results obtained by different groups using the same separation techniques (Hall, 1972) and may partly account for the different results obtained by the post-
precipitation technique in comparison with other separation techniques (Cotes, Mussett, Berryman, Ekins, Glover, Hales, Hunter, Lowry, Neville, Samols & Woodward, 1969).

ACKNOWLEDGMENTS

We are grateful to Dr B. Morgan and Dr D. P. Smith of the University Department of Chemical Pathology, Leeds General Infirmary, for their advice and for their estimation of plasma proteins, and to Dr B. Roberts for providing patients.

REFERENCES


