Binding of I-131 Labeled Thyroxine by Water Soluble Human Pituitary Proteins

The binding of thyroxine (T4) and triiodothyronine (T3) by water soluble proteins derived from human heart muscle and pituitary (WSPitP) was first reported in 1963[1]. It was then shown with the aid of paper electrophoresis (veronal as a buffer pH 8.6) that T4 is bound by protein migrating to the region of the fast serum β-globulins and T3 to the more slowly migrating β-globulin region[1]. Subsequent studies in mice revealed that water soluble proteins obtained from Thyrotropic and Mammotropic pituitary tumors[2] also bind T4 and T3 in the area corresponding to the β-globulin region of the human serum[3]. The previous studies were undertaken to investigate the binding of T4 by human tissues only and were not designed to establish whether a difference exists between human serum and human tissue in their T4 binding properties.

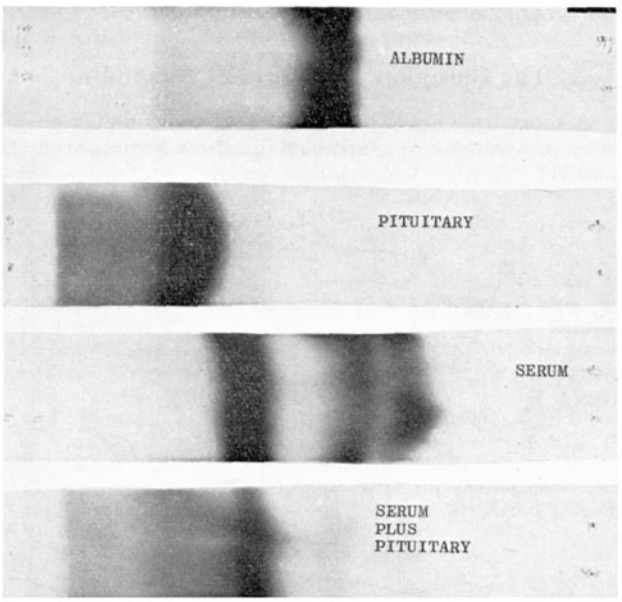
Further studies of immunologic nature revealed that pituitary and heart proteins contained antigens which reacted with β-globulin and albumin fractions of the human serum[4]. In addition, immunoelectrophoretic patterns suggested that the tissue proteins contained a component with the electrophoretic mobility of a β-globulin, or between the β- and γ-globulin of the human serum[4]. In order to identify the thyroxine binding protein of the pituitary gland (TBPitP) more studies are now being conducted; some of the more recent findings are herein reported.

In the present report the electrophoretic mobility of extracts of WSPitP was initially investigated, and next the T4 binding properties of these proteins were studied by the use of I-131 labeled thyroxine (T4*), in conjunction with paper electrophoresis and radioautographic techniques.

The human pituitaries used in this investigation were obtained at the time of autopsy and were kindly furnished to us by Dr. J. PICKREN. Normal serum and albumin used as controls were obtained from the Cutter Laboratories. Water soluble proteins from the pituitaries were extracted following a procedure previously described[2], and protein electrophoresis of WSPitP was carried out with veronal as a buffer (pH 8.6) during 16 h. The electrophoretograms thus obtained were compared with human serum electrophoretograms as a standard of mobility and revealed proteins with the mobility of albumin (20%), α-globulins (39%), βglobulin (19%) and γ-globulin (21.8%).

To study the binding capacity of the WSPitP mixtures, several amounts of T4* (0.005–0.0012 μm), specific activity 42.8 mc/mg, and constant quantities of WSPitP (100 μg) were submitted to paper electrophoresis with either veronal or Tris maleate as buffers (pH 8.6)[5] during 16 h. Mixtures of the same amounts of T4* and human serum (50 μg), or human albumin (50 μg) were used as controls of electrophoretic mobility. After electrophoresis with Tris maleate as a buffer the paper strips were dried and radioautograms made with the electrophoretograms, following the usual procedures. In the serum mixtures T4* was bound in the prealbumin, albumin and inter-α-globulin areas; in the albumin mixtures to the albumin area; and in the WSPitP mixtures to an area which coincided with the β-globulin area of the human serum (Figure). Results were similar when veronal was the buffer; the only difference being the absence of a T4* prealbumin binding in the mixtures.

The electrophoretic mobility of the TBPitP and the thyroxine binding proteins of the human serum (TBP) were also investigated, following a slight variation of the above mentioned procedures. Mixtures containing serum and T4* (A); serum, WSPitP and T4* (B); and WSPitP and T4* (C) were submitted to electrophoresis with Tris maleate as a buffer (pH 8.6) during 16 h and then to radioautography. In mixtures A and C the results agreed with those of the previous experiment. In mixture C, however, four bands of T4* binding were visible; three of them originating in the serum and one in the WSPitP (Figure). Absence of a T4* prealbumin band was found in the same mixtures when veronal was the buffer.



Radioautograms of electrophoretograms of I-131 labeled thyroxine (T4*) 0.0012 μm added to human albumin; T4* 0.0012 μm added to water soluble human pituitary proteins; T4* 0.0012 μm added to human serum; and T4* 0.005 μm added to a mixture of human serum and water soluble human pituitary proteins. Electrophoresis with Tris maleate buffer pH 8.6 during 16 h.

By the use of electrophoresis and radioautography the present investigation has confirmed the existence of a TBPitP in WSPitP. This TBPitP has an electrophoretic mobility which coincides with the β-globulin region of the human serum. More studies are now in progress to isolate the TBPitP and provide information on the chemical characteristics of this protein and its possible physiological significance.

Résumé. Etude de la propriété qu'ont les protéines pituitaires solubles dans l'eau de lier la thyroxine d'indice I-131. L'électrophorèse au pH 8,6, en utilisant comme tampons le véronal et le tris-maléate, de même que l'autoradiographie sur bandes de papier montrent que ces protéines lient la thyroxine I-131 dans une zone correspondant à la fraction des globulines β du sérum humain.

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