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THE MECHANISM OF THYROXINE TRANSFER FROM PLASMA TO TISSUE BINDING SITES

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SUMMARY

1. An examination has been made of the uptake of thyroxine by rat livers perfused with very dilute solutions of thyroxine-binding globulin (TBG) and thyroxine-binding pre-albumin (TBPA).

2. Under these circumstances the rate of transfer of thyroxine to the liver is very similar to the rate of thyroxine dissociation for both TBG and TBPA.

3. It is concluded that thyroxine transfer from plasma to tissue-binding sites involves its initial release into the free state and not, as has been suggested, direct exchange.

INTRODUCTION

In both the plasma and the tissues thyroxine is found almost entirely in the bound state. In the plasma it is bound to specific proteins (TBG and TBPA in the case of human plasma) and in the tissues most of it is adsorbed at membrane surfaces (Hillier, 1970*b*). However, there is dispute as to how the hormone transfers from one binding site to the other.

Ingbar & Freinkel (1960) proposed that only free thyroxine can be taken up by the tissues and a substantial body of evidence supports this view (e.g. Hillier, 1968*a*, *b*). This so-called 'free thyroxine theory' has recently been examined quantitatively with respect to thyroxine uptake by the liver and, in general, it has been found to provide a good model for the experimental findings (Hillier, 1969, 1970*a*). By this theory it is envisaged that, as the plasma passes through the liver, a substantial proportion of the bound thyroxine is released into the free state. This released hormone is then competed for, on the one hand by TBG and TBPA in the plasma, and on the other hand by binding sites on the liver cells. The dissociation of thyroxine from TBG and TBPA is seen therefore as an essential, initial step in transfer of the hormone from the plasma to the tissues.

Oppenheimer, Surks & Schwartz (1969) have proposed an alternative

theory in which the free thyroxine pool plays no part. Here, thyroxine is envisaged as exchanging between plasma and liver binding sites directly. As the protein-bound thyroxine in the plasma approaches a tissue binding site, the hormone is attracted from the one site directly on to the other and is never at any stage free. This has been called 'the collision theory.'

With a knowledge of the rate of thyroxine dissociation from TBG and TBPA (Hillier, 1971) it is possible to distinguish between these two theories with regard to thyroxine uptake by the liver. This tissue very avidly takes up free thyroxine (Hillier, 1969), so much so that, provided the concentration of binding protein in the perfusion fluid is sufficiently low, virtually all of the hormone present in the free-state within the sinusoids will be trapped by the cells and remain there.

If one considers the uptake of thyroxine from a very dilute solution of TBG on the 'free thyroxine theory' then the hormone, in order to transfer from the TBG to the liver, must first go into free solution (i.e. dissociate from the TBG). Consequently the transfer rate cannot exceed the rate of thyroxine dissociation from TBG, which has a $t_{\frac{1}{2}}$ of 8·1 min at 20° C (Hillier, 1971). It would be expected therefore that the rate of thyroxine transfer from TBG to liver would have a $t_{\frac{1}{2}}$ of about 8·1 min at high dilution of binding protein (where all of the thyroxine released from the TBG would be trapped by the liver). With a high concentration of binding protein in the perfusion fluid the transfer rate would be less than the dissociation rate since a substantial proportion of the dissociated thyroxine would be rebound in the perfusion fluid by the binding protein. This effect has already been observed experimentally (Hillier, 1969).

In a similar way the 'free thyroxine theory' predicts that the rate of thyroxine transfer from TBPA to liver should approach the rate of thyroxine dissociation from TBPA ($t_{\frac{1}{2}}$ 53 sec at 20° C) at high dilution of binding protein.

The 'collision theory' makes no definite predictions as to transfer rates.

METHODS

It was the purpose of these experiments to expose very dilute solutions of TBG and TBPA containing radio-thyroxine to liver cells for various periods of time. This was done by pumping perfusion fluid through the tissue at different rates and, at each rate, to measure the time the perfusion fluid was within the liver substance ('the perfusion time'). Since 80–90% of the extracellular fluid within the liver is in the sinusoids (Bradley, 1963) the perfusion time is a good approximation to the time the fluid is actually in contact with the cells. The solutions of binding protein were introduced into the system by injecting them into the perfusion stream just before the fluid entered the liver (Fig. 1). Details of this preparation and of materials and methods will be found in an earlier paper (Hillier, 1969).

The perfusion fluid was 0·8% NaCl buffered to pH 7·4 with 0·01 M phosphate buffer. All experiments were performed at room temperature (20° C). The solutions

of TBG and TBPA were the same as those used previously (Hillier, 1971). They were diluted with perfusion fluid to give a concentration of 5% of their normal plasma levels. Thyroxine labelled with ^{125}I was added to these solutions at tracer concentration and aliquots (0·1 ml.) were then injected into the perfusion stream. This latter procedure involved a further twentyfold dilution so that the final concentration of binding protein in all experiments was about 0·25% of plasma levels.

The procedure of a typical experiment. A male albino rat (350 g) was anaesthetized with a heparin-Nembutal mixture, the hepatic portal vein cannulated and the liver washed through with 30–40 ml. perfusion fluid. The liver was removed and suspended by its cannula into a bath of saline. Perfusion was switched from gravity feed (40 cm water) to a peristaltic pump and the tissue flushed with a further 30 ml. fluid at a rate of about 6 ml./min. The flow rate was then adjusted to a suitable value and the organ resuspended in a small beaker containing 25 ml. 5% plasma; the plasma was used to trap the radio-thyroxine as it left the tissue.

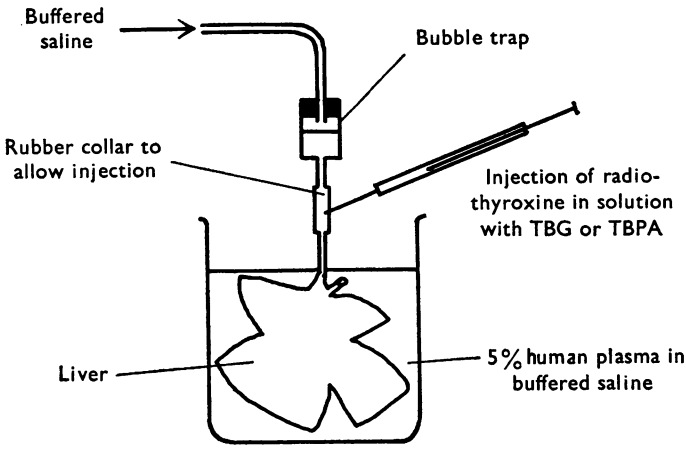

Fig. 1. The method of liver perfusion.

The perfusion time was then estimated by injecting a suspension of red blood cells into the perfusion stream and timing their passage through the tissue. The mean perfusion time was measured between injection and the time at which the bulk of the red cells was ejected from the liver into the bath.

An aliquot (0·1 ml.) of radioactive TBG or TBPA was then gradually injected over a period of time equal to the mean perfusion time. This ensured that the final concentration of TBG and TBPA was the same in all experiments and did not vary with the flow rate. The liver was then perfused for a period equal to five perfusion times to ensure complete washing through of the solution; this procedure did not, however, wash out any radio-thyroxine trapped by the liver (Hillier, 1969). The 5% plasma in the bath was then collected, made up to 50 ml. and aliquots taken for estimation of its radioactivity.

The liver was resuspended in a large bath of saline and rapidly perfused for 3–4 min before a second estimation was performed at a different flow rate. No more than five measurements were made on any one preparation and these were made in a random order of flow rates. Results between different preparations proved to be very similar and so all of the results were pooled together for presentation.

RESULTS

All of these results are illustrated in Fig. 2; the ordinate axis is on a logarithmic scale and six liver preparations were used for both the TBG and the TBPA experiments. For both protein solutions the longer the fluid spent within the liver the more thyroxine was removed from it. In both cases thyroxine uptake showed two components. Within the first few seconds a small proportion of the hormone was rapidly taken up by the

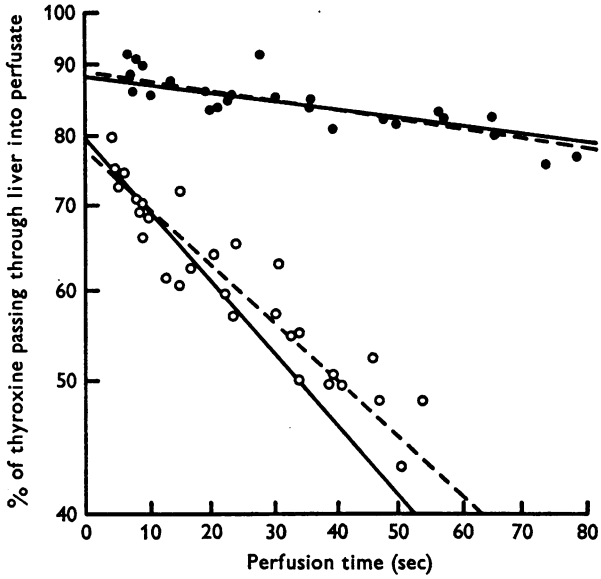

Fig. 2. The uptake of radio-thyroxine by perfused rat livers from dilute solutions of TBG (●) and TBPA (○). The amount of radio-thyroxine passing through the liver into the perfusate is plotted on a log scale against the time that the fluid spends within the liver substance. The dashed lines are regression lines drawn through the experimental points by the method of least squares. They have half-times of 7·7 min and 66 sec for the TBG and the TBPA results respectively. The continuous lines represent the rate of thyroxine dissociation from TBG ($t_{\frac{1}{2}}$ 8·1 min) and TBPA ($t_{\frac{1}{2}}$ 53 sec) at 20° C.

liver; this proportion was about 12 % in the case of the TBG solution and about 21 % in the case of the TBPA solution. Following this initial fast-uptake process the thyroxine that remained in solution was taken up much more slowly, very slowly indeed in the case of TBG.

In solution with binding protein, thyroxine is present in two forms – a small proportion in the free state and the rest bound. It seemed probable therefore that the fast-uptake of thyroxine represented trapping of the free hormone by the liver and that the slow-uptake represented trapping

of the protein-bound fraction. The same conclusion was reached in a previous study of thyroxine uptake from diluted rat plasma (Hillier, 1969).

In Fig. 2 the continuous lines drawn through the experimental points during the slow-uptake phase do not refer to the liver but their slopes represent the rate of thyroxine dissociation from TBG and TPBA at 20° C (half-times of 8·1 min and 53 sec respectively). The dashed lines in Fig. 2 are regression lines drawn through the experimental points by the method of least squares. They have half-times of 7·7 min for TBG and 66 sec for TBPA. It is clear that the rate of thyroxine transfer from binding protein to liver is very similar to the rate of thyroxine dissociation for both TBG and TBPA.

DISCUSSION

In the Introduction the 'free thyroxine theory' has been considered. The findings reported here make it likely that thyroxine dissociates from the binding protein and is then quantitatively captured by the liver cells. As the perfusion time is 10–20 % longer than the time the fluid actually spends in contact with the cells, it would be expected that the observed uptake rate would be 10–20 % slower than the dissociation rate. This could account for the small difference between the $t_{\frac{1}{2}}$ for the dissociation of TBPA of 53 sec and the observed $t_{\frac{1}{2}}$ of the slope of 66 sec (Fig. 2). The slope of the TBG results is really too shallow to allow very accurate comparison. However, considering the number of variables involved, the similarity between the experimental results and the theoretical predictions is very good.

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