UPTAKE OF TRIIODOTHYRONINE AND THYROXINE BY ISOLATED RABBIT ADIPOCYTES

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1. Introduction

The prevailing view regarding uptake of triiodothyronine (T_3) and thyroxine (T_4) by tissues is that cellular uptake is largely dependent upon the concentration of free hormone in blood and extracellular fluids [1,2]. It is also assumed that thyroid hormone reaches the cell surface as the free unbound molecule and enters sensitive cells freely by passive diffusion. Although a great deal of information exists on the interaction of T_3 and T_4 with the proteins of blood plasma, little knowledge is available on uptake of the hormone by cells.

Adipose tissue may be considered a target tissue for thyroid hormone, based on the fact that triiodothyronine enhances lipolysis in adipose tissue or free cells, especially in the presence of catecholamines [3–6]. A diminished lipolytic response to norepinephrine has also been found in adipose tissue of hypothyroid human beings [7]. In addition, adipose tissue or fat cells from hyperthyroid rats show an increased rate of oxygen consumption [5,6]. Since a relatively homogeneous preparation of fat cells can be obtained readily from adipose tissue [8], these cells can serve as a model system for an investigation of thyroid hormone transport into cells.

We have studied the uptake of T_3 and T_4 by intact rabbit adipocytes. The results indicate the existence in fat cells of high affinity, low capacity binding sites, with similar affinities for triiodothyronine and thyroxine. Since triiodothyronine was taken up by the lipid layer of lysed cells to a much greater extent than by intact cells, it is suggested that a barrier to free diffusion of thyroid hormone exists in the fat cell.

2. Materials and methods

Male white New Zealand rabbits weighing 3–4 kg were obtained from Hare Company, New Milford, NJ. For each experiment, the perirenal and epididymal fat tissue of one animal was taken to prepare cells by the method of Rodbell [8], except that ovalbumin, which binds T_3 and T_4 weakly [9], was used instead of bovine serum albumin. Fat cells were counted in quadruplicate in a Fuchs-Rosenthal counting chamber, 0.2 mm deep.

[^{125}I]T_3 (spec. act. 45.5 Ci/mmol) and [^{125}I]T_4 (spec. act. 64 Ci/mmol) in 50% propylene glycol were obtained from Abbott Laboratories.

Inorganic [^{125}I]iodide contamination of the [^{125}I]T_3 and [^{125}I]T_4 was less than 1%, as determined by paper electrophoresis. No detectable [^{125}I]T_4 was present in the [^{125}I]T_3 sample; [^{125}I]T_3 contamination of the [^{125}I]T_4 preparation was less than 1%.

To study uptake, a mixture containing $3–4 \times 10^6$ cells and [^{125}I]T_3 or [^{125}I]T_4 in 2 ml total volume Krebs-Ringer phosphate (KRP)–3% ovalbumin buffer, pH 7.4, was rotated gently in a plastic tube at 37°C. Bound radioactive hormone was determined by collecting an aliquot of the suspension on a 5.0 μm Millipore filter (SMWP 02500), washing with 0.9% NaCl and counting the air-dried filter. Results were corrected for adsorption of 1.7% and 1% of [^{125}I]T_3 and [^{125}I]T_4, respectively, to the filter in the absence of fat cells.

For uptake studies with cell lipid, the usual 2 ml incubation mixture containing avg. 3.5×10^6 cells in KRP–3% ovalbumin buffer was sonicated for 10 s (Branson Sonifier, Cell-Disruptor 200) to lyse the cells. After separating the lipid layer from the aqueous

phase by centrifugation (400 × *g* for 10 min), the aqueous phase was removed with a Pasteur pipet and replaced with fresh KRP–3% ovalbumin solution to bring the volume back to 2 ml. Following incubation with [^{125}I]T_3 at 37°C, both lipid and aqueous phases were counted separately.

3. Results and discussion

The time course of uptake of [^{125}I]T_3 and [^{125}I]T_4 by rabbit fat cells is shown in fig.1. Both iodothyronine compounds are taken up rapidly, with a small peak observed in about 1 min; the uptake falls slightly, then rises gradually, remaining essentially constant between 5 min and 10 min (fig.1). A 5 min time was selected for the uptake studies, since in this interval, equilibrium seems to be reached between hormone bound to the cells and hormone in the medium.

The percent [^{125}I]T_3 or [^{125}I]T_4 bound to the fat cells in 5 min, as a function of concentration of added hormone, is shown in fig.2. T_4 is taken up about one-half to one-third the extent of T_3, depending on the concentration (fig.2). It is reasonable to assume that binding above 1×10^{-10} M hormone concentration, where the curves level off (fig.2), represents mainly 'nonspecific' binding. A Scatchard plot of the data corrected for this nonspecific binding (2.9% and 0.4% of added T_3 and T_4, respectively) gives the results shown in fig.3. The slopes of the T_3 and T_4 binding curves (fig.3) are almost parallel, indicating

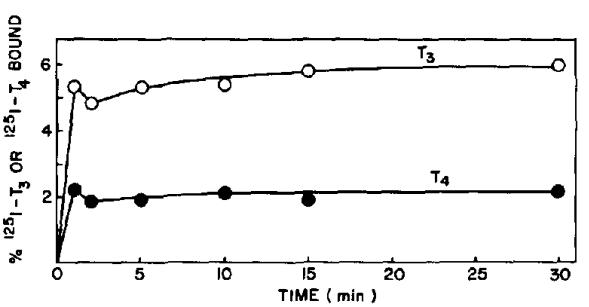

Fig.1. Time course of uptake of [^{125}I]T_3 and [^{125}I]T_4 (1.5×10^{-11} M) by intact rabbit fat cells (1.8×10^6 cells/ml), at pH 7.4 and 37°C, in Krebs-Ringer phosphate–3% ovalbumin buffer. The results are means of two separate experiments, each determination done in duplicate, using fat cells isolated from the fat tissue of two animals.

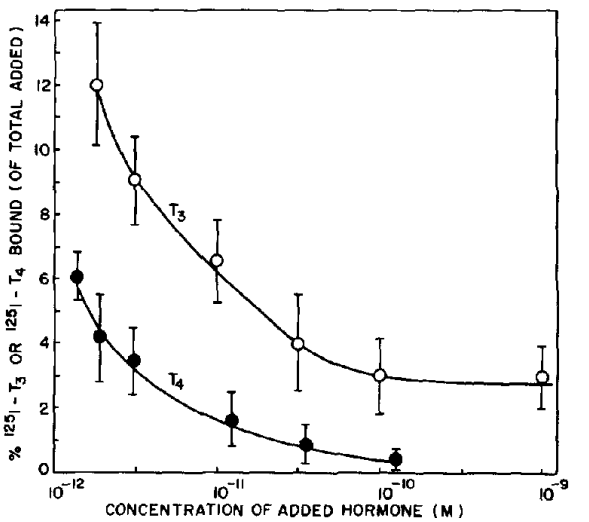

Fig.2. Uptake in 5 min of [^{125}I]T_3 and [^{125}I]T_4, at pH 7.4 and 37°C, by intact rabbit fat cells (1.8×10^6 cells/ml) in KRP–3% ovalbumin buffer, as a function of added hormone concentration. Each point is the mean (±SD) of results obtained from separate experiments with fat cells isolated from fat tissues of 3–6 animals, each determination done in duplicate.

that both compounds are bound with about the same affinity. From fig.3, the apparent association constant for T_3 interaction with its site is K 4.2 ± 0.8×10^{11} M^{-1}. For T_4, a K of 5.5 ± 1.8×10^{11} M^{-1}

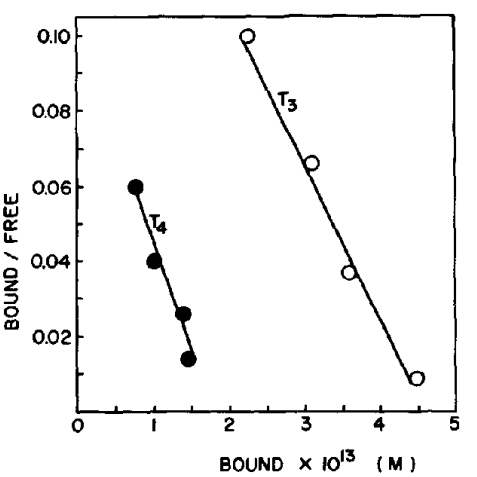

Fig.3. Scatchard plot of the binding data given in fig.2 corrected for nonspecific binding.

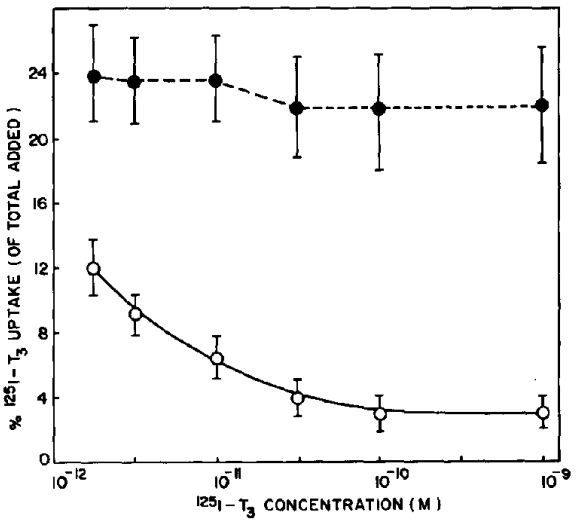

Fig.4. Uptake of [^{125}I]T_3 by the lipid layer (•), isolated from lysed cells, compared to uptake by an equivalent number of intact cells (○), at pH 7.4 and 37°C. Uptake by intact cells are results for T_3, previously shown in fig.2.

is obtained for binding to its site. The corresponding dissociation constants for T_3 and T_4 binding are 2.4×10^{-12} M and 1.8×10^{-12} M, respectively. These values are in the range of free hormone concentration in rabbit serum [10]. Extrapolations to the abscissa in fig.3 give estimated maximal binding capacities for T_3 and T_4 of 323 and 117 molecules per cell, respectively.

At present, the subcellular location and possible function of the high affinity sites remain to be determined.

Hillier [11] has shown previously that partition of the relatively hydrophobic T_3 molecule between a phospholipid layer and an aqueous phase greatly favors the lipid phase. Since there is an extremely high content of lipid in fat cells [12], it was of interest to determine the uptake of T_3 by the lipid layer present in a quantity of cells equivalent to that used in the regular uptake experiments (fig.2). As shown in fig.4, uptake of [^{125}I]T_3 by the isolated lipid layer is two to five times greater than uptake by the intact cells. This difference is not due to the 5 min uptake time, since similar results were obtained in 30 min uptake experiments (data not shown). These results indicate that fat cells have the potential to take up T_3 to a much greater extent on the basis of their lipid content alone than is actually observed with intact cells. It would appear, therefore, that some barrier exists preventing T_3 from interacting freely with cell lipid. Whether this barrier is located at the level of the plasma membrane or within the cell is not known. Nevertheless, the existence of such a barrier would argue against passive diffusion as the major transport process for entry of T_3 into the fat cell.

Acknowledgement

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