THE ESSENTIAL ROLE OF ALBUMIN IN THE ACTIVE TRANSPORT OF THYROID HORMONES INTO PRIMARY CULTURED RAT HEPATOCYTES

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1. Introduction

We have shown that both 3,5,3′-triiodo-L-thyronine (T_3) [1] and L-thyroxine (T_4) [2] are taken up by primary cultured hepatocytes of adult rats by two saturable processes and by diffusion. One saturable uptake system shows a high affinity with a low capacity while the second one has lower affinity and higher capacity. The high affinity systems of T_3 and T_4 are energy dependent in contrast with their low affinity systems [1,2]. T_4 inhibits the high affinity system of T_3 competitively [2] and vice versa (E.P.K., R.D., H.F.B., unpublished). We suggested that the high affinity systems represent a transport function while the uptake systems with low affinity may be involved in binding at the cell surface [1]. Our results were obtained from incubations of hepatocytes in a medium containing 10 g/l bovine serum albumin. Since similar studies [3] with cultured monkey hepatocarcinoma cells do not show saturable uptake of T_3 in the absence or with a low concentration of albumin (~2 g/l), we decided to study the effect of albumin on the uptake of T_3 in our system.

The results here reported show that at <5 g/l albumin, saturable uptake is not observed, in contrast with higher concentrations of albumin. By increasing the concentration of albumin from 5–20 g/l the V_{max} values of both affinity systems of T_3 increase, whereas the K_m values remain unchanged.

It is therefore suggested (a) that albumin is necessary for optimal diffusion through the unstirred waterlayer around the cell in a system of cultured rat hepatocytes in monolayer and (b) that increasing concentrations of albumin lead to augmentation of the uptake processes which are involved in transport and binding of T_3.

2. Materials and methods

The materials used and the procedure of isolation and culture of parenchymal rat liver cells has been described [1].

Incubations were performed in quadruplicate for 1 min at 37°C with [^{125}I]T_3 and increasing amounts of unlabelled T_3 in 4 ml incubation medium with varying concentrations of bovine serum albumin as mentioned in section 3. The subsequent procedure for calculating the uptake kinetics is similar to that in [1]. Free T_3 concentrations at the different albumin concentrations used were estimated with equilibrium dialysis [4]. The range of the free T_3 concentrations ([FT_3]) was 1.3 nM to 76 μM.

Correction for the contribution of diffusion was done essentially as in [5].

3. Results

3.1. *Effect of albumin on diffusion*

As illustrated in fig.1, without albumin in the incubation medium, the velocity of diffusion at a [FT_3]=22.3 μM is 6.2 (SEM=1.0) nmol . 35 μg DNA^{-1} . min^{-1}. The use of 1 g/l albumin leads to an increase ($p<0.01$) in the diffusion velocity to a level of 36.6 (± 1.7) nmol.35 μg DNA^{-1}.min^{-1},

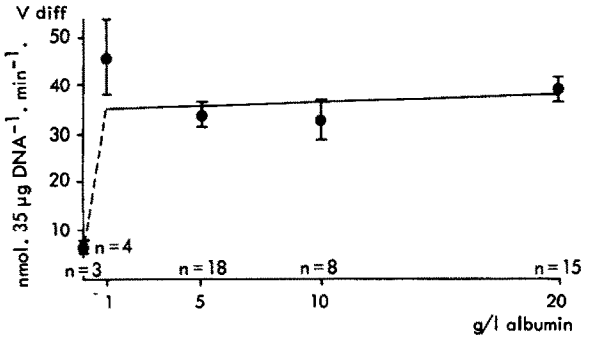

Fig.1. Effect of albumin in the incubation medium on uptake of T_3 by diffusion by primary cultures of parenchymal rat liver cells, at 22.3 µM, free T_3 at 37°C and with 1 min incubation. Each point represents the mean ± SEM of *n* experiments (each carried out in quadruplicate).

which does not increase further under conditions of higher albumin concentrations.

3.2. *Effect of albumin on the kinetics parameters of saturable uptake*

At zero or 1 g/l albumin concentration in the incubation medium the V_{max} of both affinity systems was not measurable. Increasing albumin to 5 g/l allowed the measurement of the kinetics parameters of both systems. Upon further increase to 20 g/l albumin the V_{max} of the high affinity system increased significantly from 26–103 pmol.35 µg $DNA^{-1}.min^{-1}$, while the K_m value did not change significantly (fig.2). Similar findings were obtained for the low affinity system, i.e., an increase of V_{max} from 5.8–10 nmol.35 µg $DNA^{-1}.min^{-1}$ (fig.3).

3.3. *Effect of 5 and 20 g/l albumin on the velocities of the high and low affinity systems*

In addition to the experiment in section 3.2 the velocities of the high and low affinity systems were studied with 5 and 20 g/l albumin in the incubation medium. Uptake of T_3 was measured at two free T_3 concentrations, viz. a low concentration (~12 nM) below the K_m of the high affinity uptake system of T_3 and a high concentration (~6.7 µM) above the K_m of the low affinity uptake system of T_3. The net uptake by the high affinity system was obtained by subtracting the contribution of the low affinity system from the total saturable uptake at the low free T_3 concentration. This contribution was measured at the same low free T_3 concentration by adding T_4 in sufficient amounts to block the high affinity system [1].

As is shown in fig.4 a significant increase in uptake of both the high (30.2 versus 13.7 pmol.35 µg $DNA^{-1}.min^{-1}$) and low (7.9 versus 5.9 nmol.35 µg $DNA^{-1}.min^{-1}$) affinity system is found at 20 g/l, as compared to 5 g/l albumin. Since the K_m does not change at the different albumin concentrations (see section 3.2), the differences in uptake between 5 and 20 g/l albumin represent changes in maximal velocity.

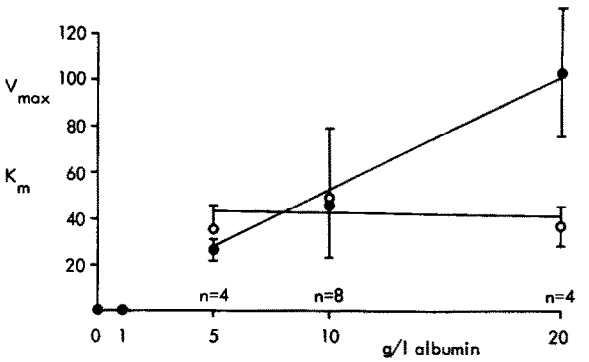

Fig.2. Effect of albumin in the incubation medium on the uptake of T_3 by the high affinity system by primary cultures of parenchymal rat liver cells at 37°C. Each point represents the mean ± SEM of *n* experiments (each carried out in quadruplicate). (•) represents V_{max} in pmol.35 µg $DNA^{-1}.min^{-1}$ (r=0.50; p<0.05) and (○) K_m in nmol/l.

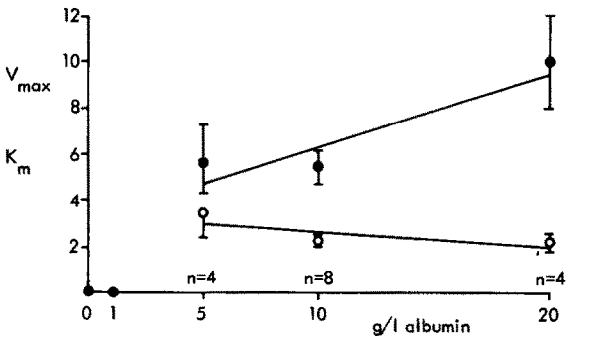

Fig.3. Effect of albumin in the incubation medium on the uptake of T_3 by the low affinity system by primary cultures of parenchymal rat liver cells at 37°C. Each point represents the mean ± SEM of *n* experiments (each carried out in quadruplicate) (•) represents V_{max} in nmol.35 µg $DNA^{-1}.min^{-1}$ (r=0.53, p<0.05) and (○) K_m in µmol/l.

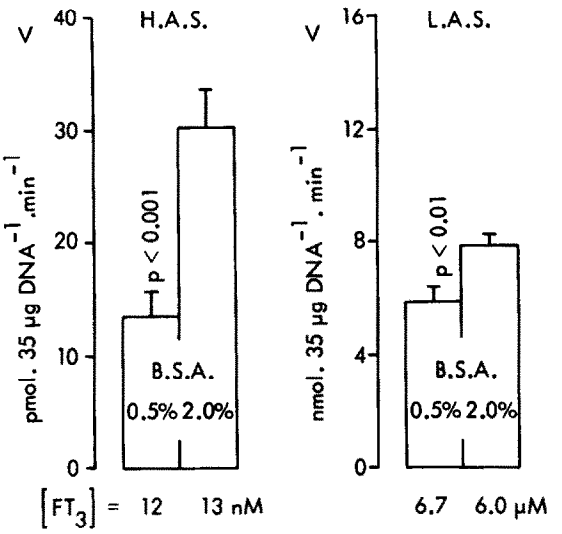

Fig.4. Effect of bovine serum albumin (BSA) in the incubation medium on the uptake velocities of T_3 by the high affinity system (HAS) (at [FT_3]~12 nM, after correction for the low affinity system, see text) and low affinity system (LAS) (at [FT_3]~6.7 µM) by primary cultures of parenchymal rat liver cells at 37°C. Each bar represents mean ± SEM of 7 experiments (each carried out in quadruplicate).

3.4. *Effect of wash procedure with 10% T_3-free serum*

After incubation of the monolayer with T_3 in 5 g/l albumin at 37°C the cells were washed with 10% T_3 free serum and results were compared with the usual procedure where incubation medium without albumin is used for washing the monolayer after incubation [1].

Discrimination of the velocities of both uptake systems was performed as described in section 3.3.

The uptake (mean ± SEM, n=8) by the high affinity system ([FT_3]=12 nM) is 14.1 ± 1.7 versus 15.6 ± 3.0 pmol.35 µg DNA^{-1}.min^{-1} (control versus wash procedure with 10% T_3 free serum) and by the low affinity system ([FT_3]=6.7 µM) 6.2 ± 0.5 versus 4.8 ± 0.6 nmol.35 µg DNA^{-1}.min^{-1} (p<0.05, paired t-test).

4. Discussion

Two binding sites with different affinities at the plasma-membrane level of hepatocytes of rats have been described for T_3 both in intact cells either in cell suspensions [6] or in primary culture [1], and in purified plasma-membranes [7] and for T_4 both in intact cells [2] and in purified plasma-membranes [8]. According to the results in this study albumin influences both diffusion and the V_{max} of the two uptake systems of T_3, without significantly affecting the K_m values. If there is no albumin present in the medium diffusion is low, but increases to a constant level when ≥1 g/l albumin is present. These findings may be explained as follows. With albumin present in the water layer around the cell, the free T_3 concentration is sufficiently stabilized to ensure optimal diffusion through the cell membrane. However, without albumin or at low albumin concentrations the water layer becomes depleted of T_3 during the uptake process so that the diffusion through the waterlayer becomes rate limiting.

At the two lowest concentrations of albumin studied (zero and 1 g/l) no V_{max} values of either uptake system could be measured, but 5 g/l albumin was sufficient to disclose both saturable uptake processes. The fact that others [3] were unable to find a saturable uptake in a similar system as we used may be explained not only on the basis of differences in cell type studied but also by the low albumin concentrations used by these investigators (maximal ~2 g/l). The observation that the K_m values of both uptake systems are not a function of the albumin concentration between 5 and 20 g/l indicates that diffusion through the waterlayer is not rate limiting [9]. The increase in V_{max} values with increasing albumin concentrations suggests that albumin has an additional role in the saturable uptake of T_3 by rat liver cells in this system. The exact mechanism by which albumin exerts this phenomenon is not known at present. A possible explanation for the change in V_{max} of the high affinity system might be the following. Removal by bovine serum albumin of fatty acids from membrane vesicles of *Escherichia coli* stimulated the active transport of proline [10]. A similar effect of albumin could stimulate the high affinity system of T_3, which is suggested to represent a transport mechanism [1].

It is noteworthy that the binding characteristics of the low affinity systems of T_3 and T_4 [2] are similar as reported for their interaction with albumin [11,12]. Therefore the low affinity system may

represent binding of T_3 to membrane-bound or water layer-trapped albumin. From unpublished observations in this study it appeared that ~0.3% of albumin remains attached to the cells after washing with incubation medium without albumin, when the monolayer was pre-incubated with 5–20 g/l albumin medium. This indicates that with increasing amounts of albumin, more albumin remains at the outer cellular surface. The increase in V_{max} as reported for this system may be explained by this increase in the amount of albumin around the cell. Against this explanation is the fact that with the highest T_3 concentrations used in our experiments albumin was not found to be saturated. (E.P.K., R.D., F. v/d Does-Tobé, unpublished) whereas the low affinity system was. Further studies are needed to clarify this problem. Washing the monolayer with medium, containing (T_3 binding) protein, after incubation lowers the uptake via the low affinity system which is in agreement with the suggestion that this system represents binding of T_3 at the cell membrane. As the high affinity system is considered to be involved in active transport through the plasma-membrane, it is not surprising that the washing procedure does not affect the amount of hormone taken up by this system.

If the postulate is correct that the high affinity system is indeed the pathway by which iodothyronines are actively transported through the plasma-membrane then it should consequently be considered that a gradient with regard to the concentrations of the free hormone exists over the cell membrane. In other words, that the intracellular free hormone concentration is higher than the extracellular, e.g., free plasma concentration. This possibility may explain the findings [13] of an ~1 order of magnitude higher affinity of T_3 for the nuclear binding site when this was measured in vivo experiments in comparison with in vitro studies. The elevated K_a in the in vivo studies may be explained by the fact that for calculation of this parameter the assumption was made that the intracellular free T_3 concentration was equal to that in the plasma. If, however, the intracellular free T_3 concentration appears to be higher than that in the plasma a lower value of the K_a will be calculated.

Acknowledgements

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