REGULATION OF THE ACTIVE TRANSPORT OF 3,3',5-TRIIODOTHYRONINE (T_3) INTO PRIMARY CULTURED RAT HEPATOCYTES BY ATP

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1. Introduction

Translocation of thyroid hormone over the plasma membrane of hepatocytes and other cells is an essential step for intracellular deiodination [1] and binding to nuclear receptors [2] and other intracellular sites [3,4]. The nucleus of the target cell is supposed to be the site of initiation of thyroid hormone action [5,6]. Recent studies show that thyroid hormone binds to isolated plasma membranes of hepatocytes [7,8] and is actively transported into hepatocytes by means of a carrier mediated process [9–11]. Evidence has been presented that T_3 and thyroxine (T_4) are translocated into the cells by different high-affinity, energy dependent mechanisms which can be blocked by ouabain [11]. This suggests that a sodium gradient over the cell membrane is essential for transport. In addition, T_3 and T_4 bind with low affinity to the plasma membrane at different sites [10,12]. Studies with human erythrocytes [13] showed similar kinetics of T_3 transport and ouabain sensitivity as our previously published observations with hepatocytes [10,11].

We reported that (1) pre-exposure of hepatocytes in monolayer to increasing amounts of T_3 results in a progressive decrease in the active transport of T_3 into the cell. The extent of this diminution is dependent on time and hormone concentration and not on *de novo* protein synthesis, as cycloheximide does not interfere with this phenomenon. (2) Pre-exposure of the cells to T_3 or fructose effected a decrease in total cellular ATP content. The positive correlation between the transport of T_3 and total cellular ATP content suggests a causative relationship. We postulate that uptake in vivo of thyroid hormone by target cells is dependent on intracellular ATP levels. In pathophysiologic conditions, such as non-thyroidal illness, fasting and hyperthyroidism, low cellular ATP concentrations may counteract further energy expenditure by decreasing cellular T_3 levels and peripheral production of T_3 from T_4.

2. Materials and methods

The procedure of the experiments and calculation of the active transport has been described [10,12]. After isolation, cells suspended in *culture* medium [10] with or without added T_3 were inoculated into plastic dishes. Four types of experiments were performed. Firstly, cells were allowed to attach to the plastic dishes without added T_3. Cells were then pre-exposed to increasing concentrations of T_3 for 15 min. Secondly, cells were pre-exposed to T_3 directly following isolation during the 4 h attachment period. Thirdly, cells were cultured for 22 h (with a change of culture medium after 7 h) in the absence of added T_3 followed by a pre-exposure period of 4 h to T_3. Culture medium normally contains 15% fetal calf serum (FCS). In this medium total T_4 concentration is 26 nM and T_3 is not detectable, however at the end of 4 h incubation due to intracellular deiodination of T_4 the medium contains 0.55 nM T_3 corresponding to a free concentration of 14 pM. The free T_3 concentration due to the addition of T_3 to this medium amounts to 0, 39, 190 and 1700 pM, respectively. In addition, medium free of T_4 and T_3 was used by replacing FCS by hypothyroid calf serum (HCS) (Rockland, Gilbertsville, PA, USA). Fourthly, after attachment, cells were pre-incubated with modified incubation medium, containing varying concentrations of glucose or fructose. After the pre-

exposure period, incubations (for 1 min, at 37°C) were performed essentially as described [10] earlier to measure the transport of T_3. The free T_3 concentration in the *incubation* medium [10] containing 1% bovine serum albumin, was 9.6 nM, which is 5- to 6-fold lower than the K_m of the transport system [10,12]. To test whether T_3 is degraded during the uptake experiments, cells were extracted with ethanol after incubation for 1 min with [^{125}I]T_3. The extract was subjected to HPLC [14] and it was found that 93% of total radioactivity eluted in the position of T_3.

For the measurement of ATP, monolayers were treated for 20 min with 2 M perchloric acid at room temperature. Supernatants were stored at −22°C until assayed by the method of Bücher [15], using the kit of Boehringer Mannheim GmbH.

Statistical evaluation of the difference with group II was performed according to the one way analysis of variance [16].

3. Results and discussion

As is shown in fig.1 (left panel) a significant progressive decrease in active transport from 21 to 11 pmol . 35 μg DNA^{-1} . min^{-1} is observed after 4 h pre-exposure of hepatocytes to free T_3 concentrations from zero to 190 pM. Similar results (fig.2, left panel) are obtained if the hepatocytes were cultured for 22 h before the 4 h pre-exposure to T_3 was started. Very similar results were obtained by adding the given amounts of T_3 to medium containing HCS (not shown).

To our knowledge this is the first report indicating that T_3 regulates its own entry into rat hepatocytes. Such a regulation is a well-known phenomenon for membrane receptors for several hormones, e.g., insulin and glucagon (for a review see [17]).

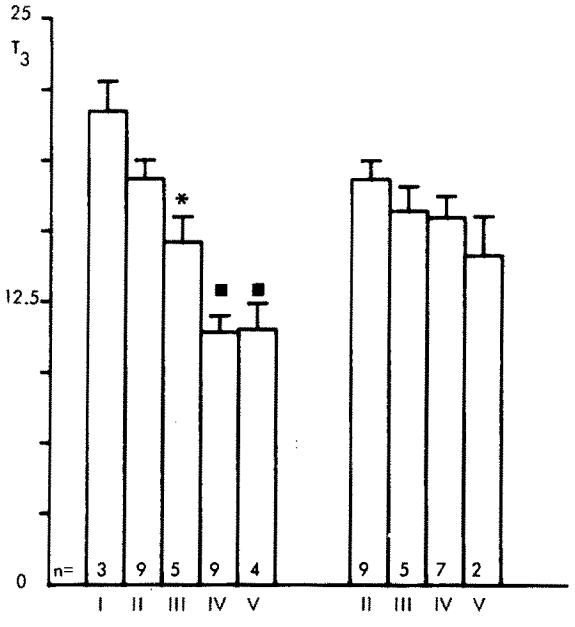

Fig.1. Effect on the active transport of T_3 (in pmol . 35 μg DNA^{-1} . min^{-1}) into primary cultured rat hepatocytes by 4 h (left panel) or 15 min (right panel) pre-exposure of these cells to increasing amounts of T_3 (medium without T_3 and T_4) (I), II-V represent free T_3 concentrations in culture medium of 0; 39; 190 and 1700 pM, respectively (see Materials and methods). Each bar represents mean ± SEM of n experiments (each carried out in quintuplicate). (* $P < 0.05$; and ■ $P < 0.001$).

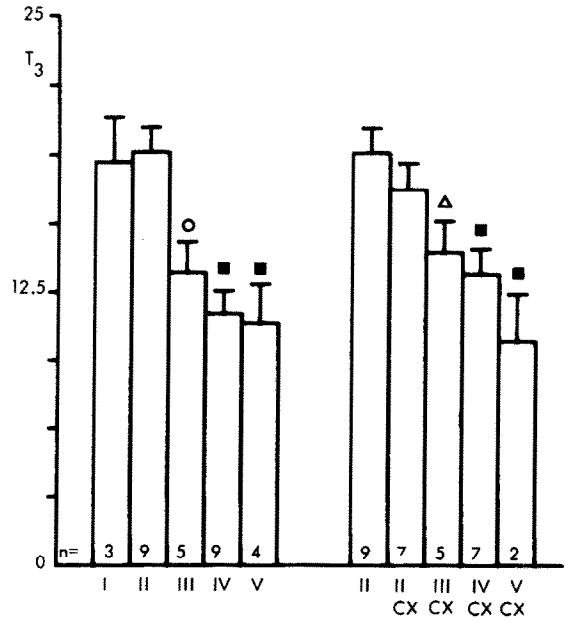

Fig.2. Auto-regulation of the active transport of T_3 in pmol . 35 μg DNA^{-1} . min^{-1} into primary cultured rat hepatocytes by 4 h pre-exposure of these cells to increasing amounts of T_3 (see legend of fig.1) in the absence (left panel) or presence (right panel) of 10 μg/ml cycloheximide (CX). This pre-exposure period followed a 22 h culture period of hepatocytes (obtained from the same rats as used in fig.1) in medium without added T_3. Each bar represents mean ± SEM of n experiments (each carried out in quintuplicate). (△ $P < 0.025$; ○ $P < 0.005$ and ■ $P < 0.001$).

To study the possible mechanism of the here described auto-regulation of T_3 entry, the following experiments were performed. Instead of a pre-exposure period of 4 h, hepatocytes were pre-incubated for 15 min with the same increasing free T_3 concentrations (fig.1, right panel). The here described auto-regulation appears to be the time dependent since an insignificant decrease (19%) is observed after 15 min compared with up to 50% after 4 h pre-exposure. This excludes a direct (e.g., conformational change of the carrier) effect at the membrane level which could lead to desensitization.

Studies with cycloheximide (fig.2, right panel) were performed to investigate whether this auto-regulation is dependent on de novo protein synthesis [18]. No effect on T_3 transport is observed in cells not pre-exposed to T_3, if protein synthesis is blocked by cycloheximide. This indicates that the turnover of the carrier is very slow with a half-life far beyond 4 h. In addition, cycloheximide does not change the effect by pre-exposure to T_3 which suggests that T_3 does not regulate its own transport by the induction of certain proteins.

Previous studies [10,11] showed that pre-incubations of the hepatocytes with metabolic inhibitors (KCN, dinitrophenol and oligomycin) blocked the transport of thyroid hormone. Under these conditions a substantial decrease of intracellular ATP concentrations has been found [10] suggesting that ATP is needed for thyroid hormone transport into hepatocytes. Since we could not find an effect by the inhibition of protein synthesis on the auto-regulation reported here we decided to study the effect of pre-exposure to T_3 on the cellular ATP content. Concomitant with the diminution in transport of T_3, a decrease of ATP is observed. A corollary of this study is shown in fig.3. A positive, statistically significant correlation is observed between the cellular ATP content and transport of T_3. If the ATP content of the hepatocytes is varied by pre-incubations with different glucose and fructose concentrations a similar relationship is found (fig.4). Since changes in intracellular ATP levels induced by separate mechanisms are associated with concomitant alterations of T_3 uptake, this strongly suggests that cytoplasmic ATP levels regulate thyroid hormone transport through the plasma membrane.

Our studies [11] involving pre-incubations of hepatocytes with ouabain suggested that thyroid hormone transport is coupled to that of sodium by $Na^+ + K^+$-

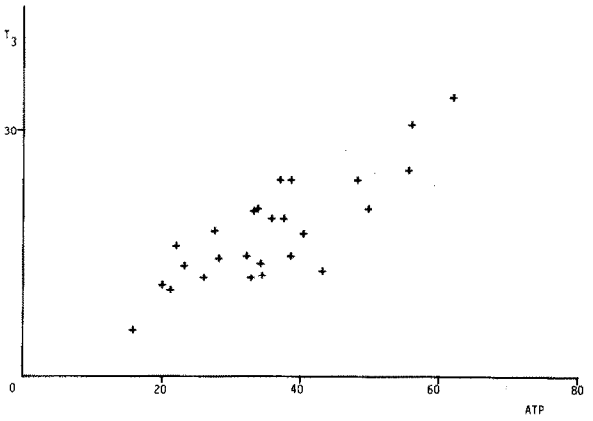

Fig.3. T_3 transport (pmol . 35 μg DNA^{-1} . min^{-1}) into rat hepatocytes in primary culture as function of cellular ATP content (nmol . 35 μg DNA^{-1}) (2 experiments; $r = 0.83$, $n = 26$, $P < 0.001$). Both parameters are varied by the pre-exposure of the cells to T_3 as described in Materials and methods.

dependent adenosine triphosphatase (Na-K-ATPase). The activity of the latter is a function of the number of enzyme units and the concentration of ATP in the cell. Both rapid [19] and long-term [20] stimulatory effects of T_3 on Na-K-ATPase have been described. This stimulation cannot be the explanation of our observations of a decreased cellular ATP content since in that case one would expect an augmented sodium gradient and therefore, increased T_3-transport.

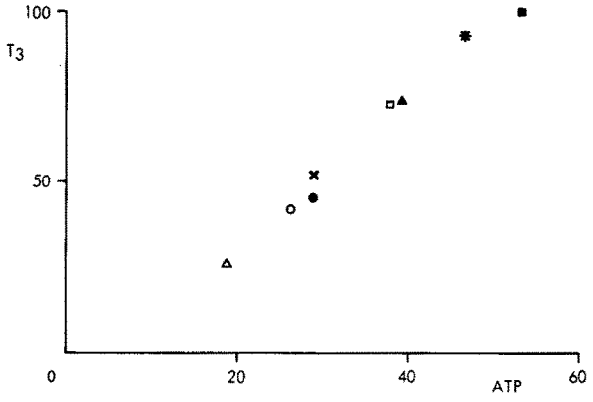

Fig.4. T_3 transport (% of control, i.e., 6.7 mM glucose) into rat hepatocytes in primary culture as function of cellular ATP content (nmol . 35 μg DNA^{-1}) (4 experiments; $r = 0.99$, $n = 8$, $P < 0.001$). Both parameters are varied by the pre-incubation of the cells with fructose (△ = 10 mM; ○ = 2 mM; ● = 1 mM) and glucose (× = 0 mM; □ = 1 mM; ▲ = 2 mM; * = 4 and ■ = 6.7 mM) as described in Materials and methods.

The here described (auto-)regulation of T_3 plasma membrane transport is observed in in vitro experiments and its significance in the homeostasis of intracellular thyroid hormone (dependent) metabolism in vivo has to be established. However, in view of decreased intracellular ATP levels in pathophysiologic conditions, a regulatory role of ATP in vivo seems likely. Hyperthyroidism can lead to a negative cellular energy balance in vivo, as determined by the reported decreased hepatic ATP contents in thyroxine injected rats [21] and running down of stores of energy rich substances in skeletal muscle of thyrotoxic patients [22]. Also, in non-thyroidal illness like shock, anemia and the diabetic state and also during fasting, decreases in cellular ATP concentrations can be found. A decrease of intracellular T_3 in these circumstances will lead to diminution of energy expenditure which may be of protective value in these states of stress. In this context it is worth mentioning that, in critically ill patients, cellular uptake of thyroid hormone appears to be diminished [23].

Low cellular concentrations of ATP will effect a decrease of intracellular T_3 concentrations in two ways. Firstly, by depressing T_3 transport into the cell and secondly by decreasing cellular production of T_3 from T_4 since T_4 uptake, being also ATP dependent ([11]), would be diminished as well.

The decreased peripheral production of T_3 from T_4 in non-thyroidal illness known as the 'low T_3 syndrome' may not only be explained on the basis of decreased concentrations of reduced glutathione which is the cofactor in the enzymic conversion of T_4 into T_3 [1], but also by reduced cellular ATP levels.

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