Vol. 145, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1987 Pages 105-110

UPTAKE OF THYROID HORMONES (L-T3 AND L-T4) BY ISOLATED RAT ADIPOCYTES

L.C. Landeta, T. González-Padrones, and C. Rodríguez-Fernández

Department of Biochemistry, Faculty of Science,
University of the Basque Country,
P.O. Box 644, 48080 Bilbao, Spain

Received April 10, 1987

3,5,3'-triiodo-L-thyronine is taken up by isolated rat adipocytes under physiological conditions by a saturable sigmoidal process, while L-thyroxine uptake follows Michaelian kinetics. Comparative studies performed with intact adipocytes and derived liposomes suggest that thyroid hormones are taken up into cells via carrier-mediated transport.

The thyroid hormones, L-thyroxine (L-T4) and 3,5,3'-triiodo-L-thyronine(L-T3), unleash the same effects at the plasma membrane level, but through different mechanisms. The different iodine contents, and the different angle formed by the α and β benzene rings in both hormones may lead to a different affinity with membrane receptors and even to a selective permeability through the bilayer [1,2]. Chemical studies carried out "in vitro" suggest that the passage of the physiologically active thyroid hormones from the external to the internal medium, depends on their structure and polarity. It would seem that both vary substantially with pH. In this complex process, the external face of the plasma membrane in contact with the incubation medium has a slightly lower pH due to the polar effects resulting from the interaction of water with the lipid bilayer [3,4].

By radioisotopic labeling and autoradiographical methods it has been shown that thyroid hormones bind to different lipoprotein cell structures, including the plasma membrane [5-18]. It has been recently suggested that L-T4 is deiodinated while passing

0006-291X/87 $1.50

through the plasma membrane. This phenomenon varies with different tissues, which could be due to their different phospholipid composition. In this way, in the liver and kidney, with dense membranes and high phospholipid contents, deiodination is high[19].

The extent of deiodination may be related to the mechanism of hormone permeability. If the thyroid hormone enters the cell by a diffusion mechanism, deiodination will affect the rate of uptake. However, if the hormone is taken up by a carrier-mediated process, the effect of deiodination on the thyroid hormone-carrier complex will be negligible[20]. The object of this work is to study the mechanism by which the thyroid hormones are taken up by the cell of rat adipose tissue.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 200-250 g fed ad libitum with standard diet were used. Rats were killed by decapitation. Samples of epididymal fat pad were incubated for a period of 1 h at 37º in a medium containing collagenase (Sigma, C.hystolyticum) and bovine serum albumin (Sigma, Fraction V) in order to obtain isolated adipocytes according to Rodbell[21]. Isolated fat cells were washed twice at room temperature with 10 volumes of a buffer solution composed of 10 mM Tris-HCl pH 7.3, 1 mM EDTA and 0.25 M sucrose. Viability of the isolated adipocytes was checked by addition of trypan blue, to a 1% (V/V) concentration, to cells suspended in "minimal medium" (10 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.3). Immediately afterwards viable adipocytes were counted under a light microscope[22].

Liposomes were prepared as follows. Lipids from the epididymal fat pad were extracted according to Rodríguez-Fernández et al.[23] and the dry extract so obtained was kept under a nitrogen atmosphere at -20º. When required, lipids were resuspended in minimal medium and sonicated at an amplitude of 10-12 µm for 90 min, at 10 s ,intervals, in an ice bath[24].

Adipocytes or liposomes suspended in the minimal medium were incubated with varying amounts of the appropriate hormones for 10 min at 37ºC in a metabolic incubator. Disappearance of thyroid hormones was measured; for this purpose hormone levels in the medium after incubation were determined by radioimmunoassay (Amersham). ^{125}I radioactivity was measured in a Gamma 4000 Beckman counter. Protein was measured according to Lowry et al.[25], with bovine serum albumin as standard.

RESULTS AND DISCUSSION

Figure 1 shows that adipocyte viability does not decrease after 20 min incubation in a medium with or without glucose (200 mg/100 ml). However, in order to ensure totally viable adipocyte populations, all the experiments presented in this communication were performed with 10 min incubation periods, in the presence of glucose.

As seen in Figure 2a, the uptake of L-T3 by isolated rat adipocytes under physiological conditions follows a saturation, sigmoidal kinetics. From these results, the corresponding Scatchard and Hill plots can be constructed (Figures 2b and 2c). The maximal uptake capacity is found to be 3.8 pmol/mg of protein, the Hill coefficient h = 2.54, suggesting positive cooperativity, and the cooperativity constant 0.96×10^{-9} M. The apparent L-T3 concentration required for half-maximal uptake (K_d) is 3.40×10^{-9} M.

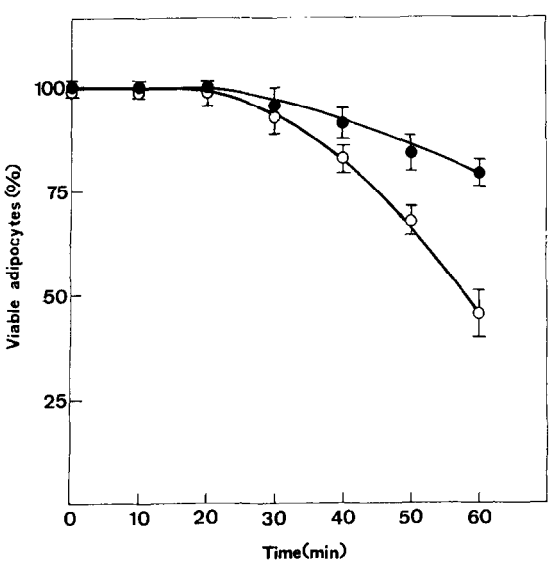

Figure 1. Viability of adipocytes isolated from rat epididymal adipose tissue, expressed as percentage of viable adipocytes as a function of incubation time. Results obtained with (●) and without (○) glucose (200 mg/100 ml) in the incubation medium. Each point corresponds to the mean ± SEM from ten values.

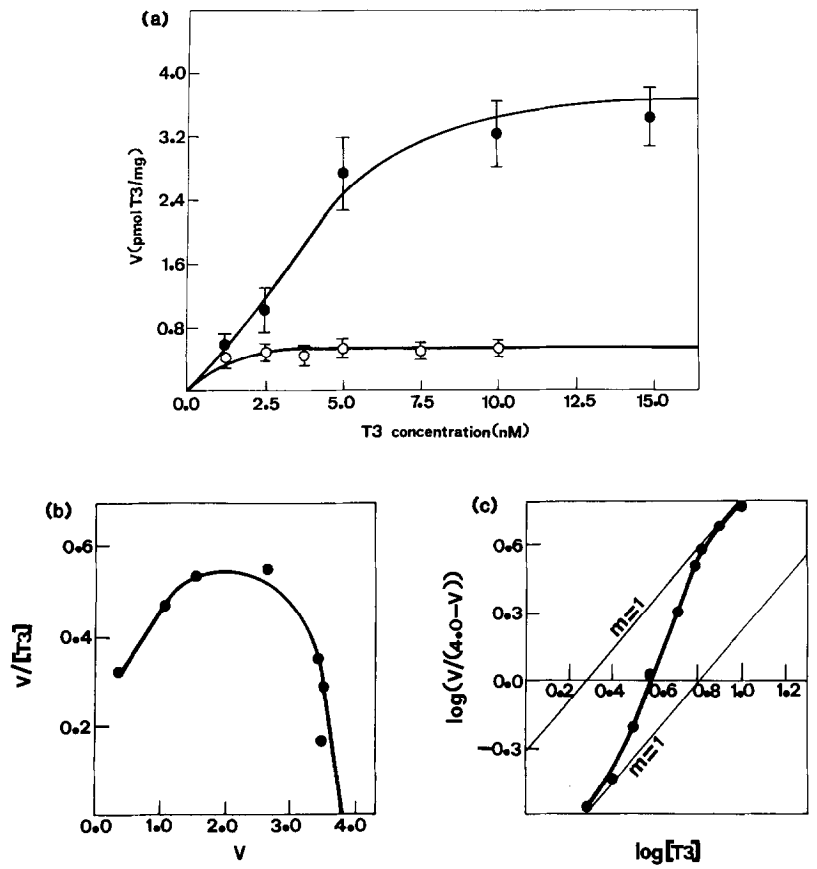

Figure 2. Uptake of L-T3 by isolated rat adipocytes. Amount of L-T3 taken up by rat adipocytes in 10 min under physiological conditions at various hormone concentrations in the incubation medium. (●) Isolated rat adipocytes; (○) Liposomes prepared from lipids extracted from the epididymal fat pad. (a) Dose-response curve, (b) Scatchard, and, (c) Hill plots. Each point corresponds to the mean ± SEM from six values. V represents the amount of hormone (pmol) taken up per mg cell protein.

Figure 3a corresponds to the uptake of L-T4 by isolated rat adipocytes under physiological conditions. It is seen to follow Michaelian kinetics. From the corresponding Scatchard plot (Figure 3b), the resulting apparent K_d value is calculated to be of 3.33×10^{-9} M, and the maximal uptake capacity of 2.4 pmol/mg of protein. From the Hill plot (Figure 3c), h is estimated to have a value of 0.96, indicating a lack of cooperativity in the uptake of L-T4 by isolated rat adipocytes.

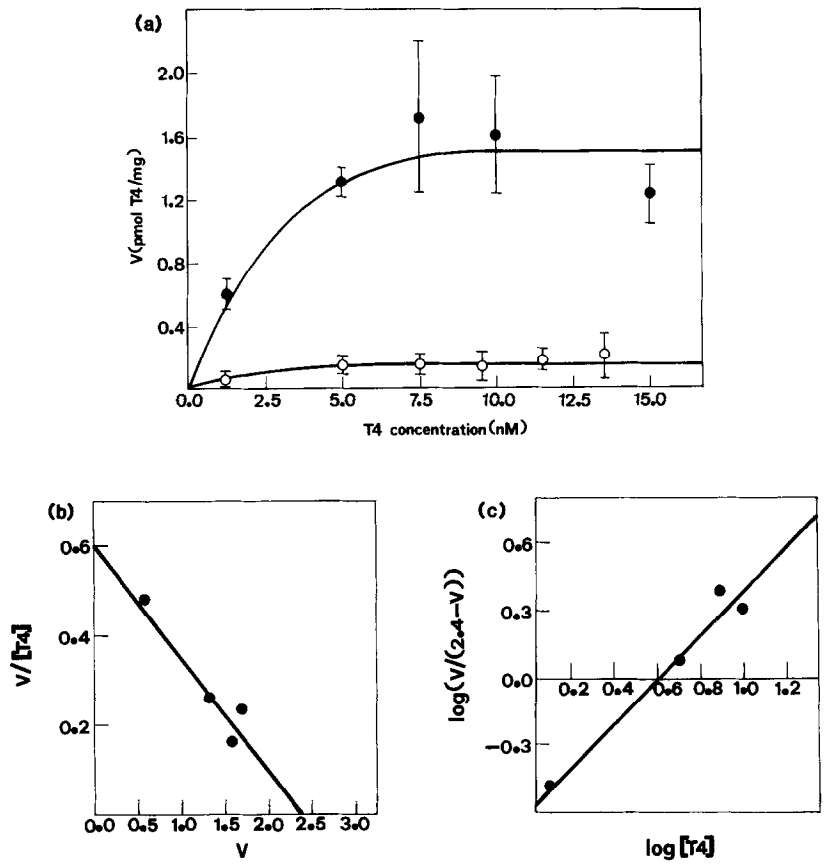

Figure 3. Uptake of L-T4 by isolated rat adipocytes. Amount of L-T4 taken up by rat adipocytes in 10 min under physiological conditions at various hormone concentrations in the incubation medium. (●) Isolated rat adipocytes; (○) Liposomes prepared from lipids extracted from the epididymal fat pad. (a) Dose-response curve, (b) Scatchard, and, (c) Hill plots. Each point coresponds to the mean ± SEM from six values. V represents the amount of hormone (pmol) taken up per mg cell protein.

From the above results it is evident that the transport of thyroid hormones, L-T3 and L-T4, into isolated rat adipocytes is not carried out by simple diffusion, but rather follows saturation kinetics. However, the transport kinetics for the two thyroid hormones are different. On other hand, it is observed that the binding of both hormones to liposomes is insignificant as compared to that obtained with viable adipocytes (Figures 2a and 3a). These results suggest that transport of L-T3 and L-T4

is mediated by carrier molecules in the plasma membrane of the adipocyte.

REFERENCES

1. Chapman, D. (1969) Structural and functional aspects of lipoproteins, pp. 36-39, Academic Press, New York.
2. Turakulov, Y.K., Tashmukhamedov, B.A. and Gagel'gans, A.I. (1979) Mitochondria, biochemical functions in the system of cell organelles, pp. 111-129, Nawka Press, Moscow.
3. Hillier, A.P. (1968) J. Physiol. 199, 151-160.
4. Hillier, A.P. (1969) J. Physiol. 203, 419-434.
5. Schwartz, H.L., Bernstein, G. and Oppenheimer, J.H. (1969) Endocrinology 84, 270-278.
6. Oppenheimer, J.H. and Surks, M.J. (1975) Biochemical action of hormones, vol. III, pp. 119-127, Academic Press, New York.
7. Turakulov, Y.K., Gagel'gans, A.I., Salakhova, N.S. and Mirakhmedov, A.K. (1975) Molecular mechanisms of action of thyroid hormones. Studies in Soviet Science, pp. 294-306, New York.
8. Rao, G.S., Eckel, J., Rao, M.L. and Breuer, H. (1976) Biochem. Biophys. Res. Commun. 73, 98-104.
9. Parl, F., Korcek, L., Siegel, J.S. and Tabacknick, M. (1977) FEBS Lett. 83, 145-147.
10. Pliam, N.B. and Goldfine, I.D. (1977) Biochem. Biophys. Res. Commun. 79, 166-172.
11. Gharbi, J. and Torresani, J. (1979) Biochem. Biophys. Res. Commun. 88, 170-177.
12. Sterling, K., Lazarus, J.H., Milch, P.O., Sakurada, T. and Brenner, M.A. (1978) Science 201, 1126-1129.
13. Krenning, E.P., Docter, R., Bernard, H.F., Visser, T.J. and Hennemann, G. (1979) FEBS Lett. 107, 227-230.
14. Krenning, E.P., Docter, R., Bernard, B., Visser, T.J. and Hennemann, G. (1981) Biochim. Biophys. Acta 676, 314-320.
15. Gharbi-Chihi, J. and Torresani, J. (1981) J. Endocrinol. Invest. 4, 177-183.
16. Segal, J. and Ingbar, S.H. (1982) J. Clin. Invest. 70, 919-926.
17. Botta, J.A., De Mendozas, D., Morero, R.D. and Farias, R.N. (1983) J. Biol. Chem. 258, 6690-6692.
18. Dozin, B., Cahnmann, H.J. and Nikodem, V.M. (1985) Biochemistry 24, 5203-5208.
19. Harris, A.R.C., Fang, S.L., Hinerfeld, L., Braverman, L.E. and Vagenakis, A.G. (1979) J. Clin. Invest. 63, 516-524.
20. Natochin, Y.V., Leontiev, V.G. and Maslova, M.M. (1975) J. Evol. Biochem. Physiol. 11, 35-42.
21. Rodbell, M. (1964) J. Biol. Chem. 239, 375-380.
22. Seglen, P.O. (1976) in Methods in Cell Biology, vol. XIII, pp. 29-83, New York.
23. Rodríguez-Fernández, C., Marino, A., Fedriani, J.R. and Macarulla, J.M. (1979) An. Quim. 75, 419-421.
24. Alonso, A., Villena, A. and Goñi, F.M. (1981) FEBS Lett. 123, 200-204.
25. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.