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TRIGGERING BY THYROTROPIN OF AN INCREASED SYNTHESIS OF TRIGLYCERIDES IN CULTURED HUMAN THYROID CELLS.

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SUMMARY : Addition of thyrotropin to cultured human thyroid cells induces a marked increase of the incorporation of (1,3-^{3}H)-glycerol and (1,2-^{14}C)-acetate in the triglycerides. The presence of thyrotropin in the medium does not modify the synthesis of phospholipids from glycerol; however, it may perhaps slightly decrease the incorporation of radioactive acetate in the phospholipids and in cholesterol. The specific radioactivity of the triglycerides remains unchanged after thyrotropin stimulation and the triglycerides'cell content is accordingly greatly increased.

Cultured thyroid cells isolated from human non-toxic goiters have been widely used to study the effect of TSH[+] on various biochemical events occuring in the thyroid cells. So far as we know the action of the latter hormone on lipid metabolism of cultured thyroid cells has not been investigated.

However, changes in phospholipid resulting from addition of TSH to slices of thyroid tissue have been reported (1); moreover, a role has been ascribed to phospholipids during the process of TSH stimulation (2,3).

In the present work cultured human thyroid cells were used to study the influence of TSH on the incorporation of labeled acetate and glycerol into various lipid classes. The data show that TSH markedly increased the synthesis of triglycerides and consequently the triglycerides content of the thyroid cell.

MATERIAL AND METHODS

Human diffuse non-toxic goiters were used immediately after operation, dissociated and cultured as described previously (4). The TSH and the radioactive precursors were added from the start of the culture to the culture medium at the following concentrations : 100 mU/ml for TSH, 43 nmoles (2.5 µCuries)/ml and 7

[+]Abbreviations : TSH, thyrotropin, TG, triglycerides.

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nmoles (12.5 μCuries)/ml for (^{14}C)-acetate and (^{3}H)-glycerol respectively. In some experiments, both precursors were added simultaneously in the same medium.

Lipids were extracted from the sedimented cells and separated from non lipids by the procedure of Folch et al. (5).

Thin layer chromatography on precoated silicagel plates was as described (7), using light petroleum ether/diethyl ether/acetic acid, 80/20/1 by volume as solvent system. Individual lipids were revealed by spraying with primuline (for radioactivity measurements or fatty acid analysis) or by the copper acetate reagent for photodensitometric measurement (6,7).

The free cholesterol was estimated by the sensitive photodensitometric method using the flying spot Vitatron TLD 100 photodensitometer (6,7). Triglycerides measurement ensued from quantitative gas liquid chromatographic analysis of the fatty acid methyl esters after transesterification in anhydrous methanolic HCl as described previously (8). Lipid phosphorus was estimated according to Bartlett (9) after perchloric acid digestion and using ascorbic acid as reducing agent.

After detection with primuline the powder corresponding to various lipids was scrapped off directly into scintillator vials and the radioactivity was measured using the Packard-Tricarb model 3380 Scintillating counter.

(1,2-^{14}C)-sodium acetate (58.6 μCuries/μmole) and (1,3-^{3}H)-glycerol (1.8 μCurie/mmole) were supplied by Amersham (U.K.) In order to compare the TSH treated cell to the untreated control, all results were related to the free cholesterol content of the preparations. As most of the free cholesterol is located in the plasma membrane of the thyroid cell (10,11) and as the cultured cells do not significantly vary in size from each other, the free cholesterol content can be considered as fairly representative of the size of the population.

RESULTS AND DISCUSSION

The data of table I show that the (^{3}H) from labeled glycerol was almost entirely recovered in the glycerides while the (^{14}C) of labeled acetate was found mainly in glycerides and in cholesterol. This table also shows that when TSH was added to the medium, the percentage distribution of both precursors in the triglyceride fraction was markedly increased.

Wide variations in the range of incorporation into given lipids from one experiment to another were observed and attributed to the inherent variability of human material. The action of TSH on lipid metabolism is more clearly showing when the precursor incorporation into various lipids in the presence of TSH, is expressed as percent of control values (no TSH added).

As shown in table II, a slightly increased incorporation of glycerol in total lipids was observed resulting mostly from an increase of glycerol incorporation in triglycerides. Radioactive free fatty acids and cholesterol contribute for only a

TABLE I. Percentage distribution of labeled precursors in four classes of lipids in the thyroid cells in culture. Influence of TSH addition to incubation medium.

	(^{3}H)-glycerol (n=4)		(^{14}C)-acetate (n=5)	
Lipid class	Control	TSH	Control	TSH
P-lipid	89.05 ± 2.7[a]	76.2 ± 3.9[b]	59.8 ± 4.2	54.7 ± 2.8
Cholesterol	2.1 ± 1	2.4 ± 1	27 ± 6	17.9 ± 2.3[c]
Fatty acids	0.1 ± 02	0.1 ± 02	2 ± 0.2	2.3 ± 0.3
Triglycerides	8.5 ± 1.3	21 ± 3.3[b]	8.5 ± 1.1	23.2 ± 2.5[b]
Esterified cholesterol	-	-	1.5 ± 0.2	2.2 ± 0.4[c]

[a] SEM
[b] difference with control significant for $P < 0.01$
[c] difference with control significant for $P < 0.05$

very small percentage to the total radioactive lipids (table I) and their percentage distribution remains unchanged under TSH stimulation (table I); therefore, the figures for cholesterol and free fatty acids reported in table II are probably devoided of significance. The radioactive acetate incorporated into fatty acids either by de novo synthesis or by chain elongation can

TABLE II. Influence of TSH on the incorporation of radioactive precursors into lipids of thyroid cells in culture. (Results are expressed as % of control values).

Lipid class	(^{3}H)-glycerol (n=4)	(1,2-^{14}C)-acetate (n=4)	(^{3}H/^{14}C) ratio
P-lipids	103.4 ± 8.5[a]	85.0 ± 7.9	145.8 ± 7.1
Cholesterol	142.2 ± 13.9	68.9 ± 11.9	186 ± 41.5
Fatty acids	186.5 ± 41.7	108.1 ± 12.9	n.d.
Triglycerides	302.3 ± 39.1	269.7 ± 37.4	101 ± 19.4
Esterified cholesterol	114.8 ± 6.1	117.2 ± 8.4	n.d.
Total lipids	120.65± 8.4	93.8 ± 7.6	-

[a] SEM

further enter the glycerides molecules during the esterification of a diglyceride or glycerophosphate (de novo synthesis) or of a lyso-P-lipid resulting from the interplay of phospholipases and acyltransferase activities. Table II indicates that addition of TSH does not increase the overall incorporation of acetate into total lipids and may perhaps slightly decrease its incorporation into cholesterol and phospholipids; on the other hand, the hormone greatly stimulates the entry of acetate into the triglycerides. No changes in the ($^{3}H/^{14}C$) ratio could be noticed in triglycerides.

As shown in table III, addition of TSH to the culture medium greatly modifies the lipid composition of the thyroid cell. The cholesterol/P-lipid ratio remains unchanged after TSH stimulation. However, the amount of triglycerides strikingly increased in the TSH treated thyroid cell, as shown by the increased values of the TG/cholesterol and TG/P-lipid ratios in the hormone stimulated cells. This increase in the thyroid cell triglycerides is undoubtebly the result of an acceleration of the de novo synthesis of the latter molecules from small building blocks such as acetate and glycerol for in three experiments with radioactive precursors the specific radioactivity of the triglycerides from the TSH treated cell was found to be fairly similar to that of the triglycerides from the control cell. The triglycerides labeled with (^{14}C)-acetate and extracted from the stimulated cell were found to display the same specific radioactivity than that of triglycerides recovered from the control cell (101.5 $\pm$ 5.3 %

TABLE III. Effect of TSH on the lipid composition of thyroid cells in culture.

TG/cholesterol molar ratio (n=5)		TG/P-lipid nmole/µg P (n=2)		Cholesterol/P-lipid µmole/µg P (n=2)	
Control	TSH	Control	TSH	Control	TSH
0.223 $\pm$ 0.5[a]	0.418 $\pm$ 0.4[b]	4.21	7.32	0.014	0.014

[a] SEM

[b] difference between TSH and control significant at a $P < 0.01$ level.

of the control value). However, the triglycerides labeled with (^{3}H)-glycerol in the TSH treated cell might have a slightly higher specific radioactivity than that of the triglycerides synthesized in the control cell (162 ± 35.9 % of control value). In thus appears that the striking increase of the triglycerides content in the TSH stimulated cell is due to an increased synthesis of the triglycerides.

The biological significance of this spectacular effect of TSH in the thyroid cell remains to be explained. Cell's properties are greatly influenced by the lipid composition of its membranes particularly the plasma membrane. Reports can be found in the recent literature on the role of the triglycerides in the ability of tumour cells to resist humoral immune attacks (12,13); it has been shown indeed that the incubation of tumour cells with hormones which decreases their sensitivity to humoral killing stimulates the incorporation of newly synthezised triglycerides into the plasma membrane thereby modifying its fluidity.

Whether or not a similar phenomenum would be involved in the process of TSH stimulation remains an open question; we feel however, that the triglyceride effect of the TSH in vitro is a provocating finding which deserves further investigation.

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REFERENCES

1. Rabinowitz, J.L. and Tavares, C.J. (1977) Biochem. J., 168, 155-160.
2. Yamashita, K. and Field, J.B. (1973) Biochim. Biophys. Acta, 304, 686-692.
3. Schneider, P.B. (1974) J. Clin. Endocrinol. Metab., 38, 148-150.
4. Winand, R.J. and Kohn, L.D. (1975) J. Biol. Chem., 250, 6534-6540.
5. Folch, J., Lees, M. and Sloane-Stanley, G.H. (1975) J. Biol. Chem., 226, 497-509.
6. Fewster, M.E., Burns, B. and Mead, J.F. (1969) J. Chromatog., 43, 120-126.
7. Calberg-Bacq, C-L., François, C., Gosselin, L., Osterrieth, P.M. and Rentier-Delrue, F. (1976) Biochim. Biophys. Acta, 419, 458-478.

8. Gosselin-Rey, C., Gosselin, L., Calberg-Bacq, C-M., François, C., Kozma, S., Osterrieth, P.M. and Van Dessel, G. (1980) Eur. J. Biochem., 107, 25-30.

9. Bartlett, G.R. (1959) J. Biol. Chem., 234, 466-468.

10. van Hoeven, R.P. and Emmelot, P. (1972) J. Membrane Biol., 9, 105-126.

11. Yamashita, K. and Field, J.B. (1974) In Methods in Enzymology (Fleischer, S. and Packer, L., eds.), XXXI, pp. 144-149, Academic Press, New York.

12. Schlager, S.I., Ohanian, S.H. and Borsor, T. (1978) J. Immunol., 120, 472-480.

13. Schlager, S.I and Ohanian, S.H. (1980) JNCI, 64, 943-950.