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CHRONIC REGULATION BY THYROTROPIN OF ARACHIDONIC ACID INCORPORATION IN CHOLESTERYL ESTERS OF CULTURED THYROID CELLS

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SUMMARY : During short term incubations, radioactive arachidonic acid and palmitic acid were incorporated in the cholesteryl ester fraction of the lipids of cultured thyroid cells. Three times more arachidonic than palmitic acid was incorporated and the incorporation of both was dependent upon the culture conditions : the presence of 1 mU/ml thyrotropin in the culture medium during four days almost completely inhibited the subsequent incorporation of the two fatty acids in the cholesteryl ester fraction whereas the total cholesterol and cholesteryl ester content of the cells was not affected.

It is now established that in most tissues the synthesis of icosanoids is controled by the availability of their common precursor, free arachidonic acid, which must be liberated from cellular lipids. The liberation of arachidonic acid under acute TSH[1] stimulation was thus suggested in cultured thyroid cells (1) or isolated follicles (2). In contrast, we had previously shown that TSH chronically depressed prostaglandin E_2 synthesis in cultured thyroid cells (3). Moreover, we reported recently that, in our hands, TSH was unable to elicit the liberation of arachidonic acid whose incorporation in cellular lipids was not modified by the presence of TSH in the culture medium (4).

In the present work we have studied short term incorporation of (^{14}C)-arachidonic acid in the CE[1] fraction. Although it represented not more than 5-6 % of the total incorporated radioactivity, this incorporation was found to be chronically regulated by TSH.

[1] Abbreviations : TSH, thyrotropin. MEM, minimum essential medium. PBS, phosphate buffered saline. CE, cholesteryl ester. TG, triglycerides. TLC, thin layer chromatography.

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Numerous reports have demonstrated the hormonal regulation of cholesteryl ester turnover in different tissues (5-9-), but this is the first report showing that (i) cholesteryl arachidonate may be one of the compounds involved in the regulation of arachidonic availability and (ii) CE turnover can be modified by TSH in cultured thyroid cells.

MATERIAL AND METHODS

Thyroid cells were obtained as previously described (3) and incubated in polystyrene Petri dishes at 37°C in a 5 % CO_2-95 % air, water saturated atmosphere in the absence (control cells) or in the presence (TSH cells) of 1 mU TSH/ml. The cells were cultured in Eagle minimum essential medium (MEM) supplemented with 10 % newborn calf serum (Gibco).

After 1,2,3 or 4 days, the cells were collected, washed and resuspended in MEM ($3x10^6$ cells/ml) and incubated with (^{14}C)-arachidonic acid (Amersham, 56 mCi/mmole, 0.04 µCi/0.5 ml suspension) or (^{14}C)-palmitic acid (NEN 53 mCi/mmole, 0.04 µCi/0.5 ml suspension).

After 30 minutes of labelling the cells were washed twice with phosphate buffered saline (PBS : NaCl 136 mM, KCl 2.7 mM, NaH_2PO_4 3.2 mM, KH_2PO_4 0.73 mM, $CaCl_2$ 0.24 mM, pH 7.2) containing 0.2 % w/v of fatty acid free albumin (Sigma) and 2.77 mM glucose.

For one assay 0.5 ml cell suspension was put in 1 ml PBS and the cellular lipids extracted with 5 ml chloroform/methanol (2:1 v/v). The lipids were separated on precoated TLC plates (Merck) in solvent "C" (hexane/ethyl ether/formic acid, 80:20:3 v/v). Standard phospholipids, glycerides, cholesterol and esterified cholesterol (all from Sigma) were run on the same plate on separate lanes and revealed with iodine vapor. The corresponding areas were scrapped off and counted with 10 ml scintillation fluid. Triglycerides and cholesterol were assayed, after TLC separation on dried eluates using commercial reagents as suggested by Ott et al (10) for cholesterol determination in lipid extracts. The reagent for the triglycerides assay (Triglycerides G-test) was from Wako Chemical and did not necessitate the addition of detergent. For the assay of cholesterol 0.5 % Triton X-100 was added to the reagent from Technicon (SMA II reagent). Esterified cholesterol content was evaluated in the eluate from cholesterol esters spots after chemical hydrolysis of the esters (Methanolic KOH, 40°C one hour).

RESULTS AND DISCUSSION

As reported in (4), thyroid cells rapidly incorporate (^{14}C)-arachidonic acid. The distribution profile of radioactivity among the cellular lipids after short term (30 min) labelling was not influenced by the presence of TSH in the culture medium. Moreover identical results were obtained whether the experiments were performed on day 1 or on day 4.

To further specify the radioactivity distribution, especially in the neutral lipids, the lipid extracts of TSH and control cells labelled on day 4 were separated on TLC plates in solvent "C" (see "method"). As

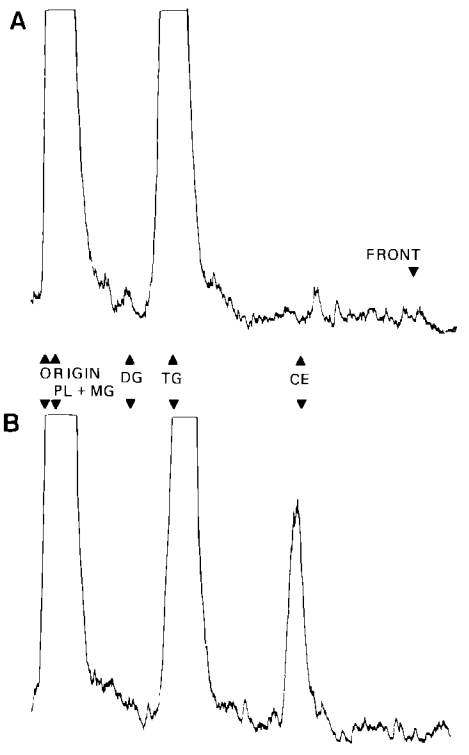

Figure 1. Radiochromatogram of ^{14}C-labeled lipid fractions of thyroid cells in culture. TSH cells (part A) or control cells (part B) were incubated with (^{14}C)-arachidonic acid at day 4. See Methods for details. Standard lipids were spotted on the same plate on separate lanes. PL : phospholipids, MG : monoglycerides, TG : triglycerides, CE : cholesteryl esters. The radioactivity on the plate was scanned using a Packard 7220 Scanner.

evidenced in Fig. 1 for a typical experiment, (^{14}C)-arachidonic acid was incorporated in the CE fraction of control cells whereas this fraction was practically not labelled in TSH cells.

When the labelling of the CE fraction was evaluated as the percentage of radioactivity in the neutral lipids (i.e. CE/CE+TG[1]), control cells and TSH cells incorporated respectively 19.9±6.2 % and 1.7±0.7 % (mean ± SE, 5 experiments) of labelled arachidonic acid in the CE fraction of their neutral lipids.

The evolution of this phenomenon was investigated during the first three days of culturing. The data presented in Table 1 show a progressive augmentation of the CE labelling capacity in control cells whereas it

Table 1. Influence of time in culture on the incorporation of (^{14}C)-arachidonic acid in the lipid fractions of thyroid control cells.

	Day 1	Day 2	Day 3
Phospholipids (cpm)	15 010	13 250	10 100
Triglycerides (cpm)	5 920	6 510	5 120
Cholesteryl esters (cpm)	60	350	640
$\frac{\text{Cholesteryl esters}}{\text{neutral lipids}} \times 100$	1.0	5.4	12.6

Cells were cultured during 1, 2 or 3 days in the absence of TSH, then collected, washed and incubated for 30 minutes in the presence of (^{14}C)-arachidonic acid as described in "Methods". The lipid extracts were separated in solvent "C". Data are expressed as cpm in the lipid fraction after TLC separation for one typical experiment.

remained at a low level in TSH cells (not shown). As shown in Table 2 this phenomenon is not a consequence of a cholesterol or CE content increase in control cells.

The incorporation of (^{14}C) palmitic acid, which is not a precursor of icosanoids, was also investigated. The total incorporation of this fatty acid was, in the same experimental conditions, three times less than that of arachidonic acid. But again control cells incorporated ten times more radioactivity in the CE fraction of their neutral lipids (13.6±4.5 %) when compared to TSH cells (1.4±4 %, mean ± SE, 3 experiments).

The results presented here demonstrate that CE turnover can be depressed by a chronic exposure to TSH in cultured thyroid cells. A depres-

Table 2. Triglyceride, cholesterol and cholesteryl ester content of cultured thyroid cells.

	Control cells	TSH cells
Triglycerides (µg)	13.4 ± 8.8 (n=4)	12.8 ± 6.6 (n=4)
Cholesterol (µg)	36.6 ± 2.4 (n=4)	36.0 ± 1.6 (n=4)
Cholesteryl esters (µg)	0.60 ± 0.15 (n=2)	0.70 ± 0.20 (n=2)

The chloroform/methanol extract of one dish ($20x10^6$ cells at day 4) was fractionated and each fraction was assayed in triplicate as described in Methods. (n=number of experiments).

sive effect of TSH on PGE_2 synthesis had previously been observed in similar conditions (4) and a possible role for cholesteryl arachidonate in icosanoid synthesis may then be considered. Indeed, though cholesteryl palmitate was also specifically generated in control cells, cholesteryl arachidonate could be a preferred substrate for CE hydrolase as recently described in rat adrenal homogenates (13). Hormonal regulations of CE turnover have been demonstrated in various tissues (5,6,7,8,12) but it always resulted in a net CE synthesis or degradation. We observed a different phenomenon since the cellular CE content was unaffected by the presence (or absence) of TSH in the culture medium.

Moreover several days were required for the CE labelling capacity to be raised in the absence of TSH (Table 1). Meanwhile, thyroid cells reorganize in tridimentional polarized structures with the baso-lateral area facing the medium in TSH cells whereas control cells exhibit "inside-out" structures where only the apical pole is accessible from the medium (14). Since an asymetry of cholesterol distribution in the plasma membrane as well as in the intracellular membranes of polarized cells has been reported (15,16), our results might be a consequence of the differences observed in the orientation of cell polarity rather than a direct effect of TSH.

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