Uptake of ^{201}Tl into Primary Cell Cultures from Human Thyroid Tissue Is Multiplied by TSH

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According to current guidelines, ^{201}Tl scintigraphy aiming at the detection of iodine-negative metastases of differentiated thyroid carcinoma is usually performed during hormone replacement in thyroid-stimulating hormone (TSH)–suppressive doses. The aim of this study was to determine the effect of TSH on thyroid ^{201}Tl uptake in vitro. **Methods:** Adherent monolayers of human thyroid tissue (1.965–3.000 million cells) were cultured after mechanical disintegration and enzymatic digestion by neutral protease. The samples were derived from patients undergoing surgical treatment of nodular goiter. Studies were performed on paranodular tissue components, exclusively. Cells originating from identical tissue samples were dispensed into matched-pair cultures and incubated in parallel by a TSH-free medium and by a medium containing 10 IU/L bovine TSH. Thyroglobulin (Tg) accumulation was calculated after repetitive measurements of Tg concentrations by radioimmunoassay. Uptake studies were initiated by adding fresh medium containing ^{201}Tl (8.2–91.0 kBq). After 1 h of incubation, the media were removed and the cells were detached by trypsin and collected by centrifugation. Uptake was measured by a γ-counter, and cellular uptake values were calculated as percentages of total activity normalized to 1 million cells. The statistical significance of differences in Tg release and ^{201}Tl uptake was corroborated in a generalized estimating equations analysis taking the variability of unbalanced replicate measurements into account. **Results:** Cells cultured in the presence of TSH displayed a 2-fold release of Tg (12.949 ng/h/million cells vs. 6.049 ng/h/million cells, $P = 0.001$) and triplicate ^{201}Tl uptake (0.718%/million cells vs. 0.249%/million cells, $P = 0.0002$). **Conclusion:** ^{201}Tl uptake in human thyroid cells is significantly increased by TSH. These data suggest that withdrawal of thyroid hormone substitution has the potential to improve the sensitivity of ^{201}Tl scintigraphy for detecting thyroid remnants or cancer recurrences. This suggestion should be further investigated in patients and in cell cultures from thyroid carcinomas.

Key Words: ^{201}Tl; differentiated thyroid carcinoma; thyroid; thyroid-stimulating hormone; thyrotropin

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Thyroglobulin (Tg) as a specific product of functionally differentiated thyrocytes is an accurate tumor marker in the clinical follow-up of thyroidectomized patients with differentiated thyroid carcinoma. The elevation of the Tg concentration in patients' sera sensitively and specifically reflects progression of differentiated thyroid cancer (*1,2*). When Tg levels indicate the onset of recurrence, it is crucial to identify the site of Tg production. The procedure of first choice is whole-body scintigraphy after administration of ^{131}I. However, a frequently observed complication in progressing thyroid carcinoma is loss of the ability of metastases to concentrate iodine. This loss makes the recurrence undetectable and also untreatable by radioiodine. For the scintigraphic detection of iodine-negative metastases, several alternative radiopharmaceuticals are in clinical use, one of which is ^{201}Tl.

Whereas it is generally accepted that endogenous stimulation of thyroid-stimulating hormone (TSH) induced by withdrawal of hormone replacement has to precede radioiodine scintigraphy, alternative radiopharmaceuticals such as ^{201}Tl are generally administered during ongoing medication in TSH-suppressive doses (*3*). The aim of this study was to elucidate the effect of TSH on the uptake of ^{201}Tl into primary cell cultures of human thyroid tissue.

MATERIALS AND METHODS

Preparation of Human Thyroid Primary Cell Cultures

Thyroid tissues were obtained from 7 patients undergoing partial thyroidectomy as a treatment of benign nodular goiter. The specimens were stored in 50-mL tubes in phosphate-buffered saline during transportation. Isolation of cells was initiated immediately after arrival in the laboratory. All preparations were performed under sterile conditions in a laminar airflow bench. All plastic materials were sterile and made for a single use.

After 15 min of washing in a rotating vessel, connective tissue and nodular structures were separated from macroscopically regular tissue components and discarded. For cultivation, regular thyroid parenchyma was selected, exclusively. The isolation procedure was performed by a combination of mechanical disintegra-

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tion and enzymatic digestion according to a protocol published by Morgan and Darling (*4*).

The tissue samples were minced into small pieces by fine surgical forceps and scissors. The tissue fragments were collected in a tube containing grade II neutral protease from *Bacillus polymyxa* (Dispase II, 5 mL/g tissue, >2.4 U/mL; Roche Diagnostics Corp., Indianapolis, IN). Enzymatic action was maintained over 30 min in the rotating tube, finally yielding a suspension of isolated cells. The suspension was filtered by a monofilament polypropylene mesh with a pore size of 250 μm (neoLab, Heidelberg, Germany) to retain connective tissue components. The filtrate was aspirated by a sterile syringe through a long injection cannula with a large lumen (Supra, 1.00 × 60 mm; Misawa, Tokyo, Japan) and ejected again with high pressure to further triturate cell aggregations. The resulting cells suspended in Dispase II were centrifuged for 10 min at 500*g*. The supernatant Dispase II was removed by an exhaust pump. The cell pellet was resuspended by 10 mL of a culture medium based on Ham's F12 solution (Gibco, Karlsruhe, Germany), supplemented by 5 hormones or growth factors (10 mg/L insulin [Gibco], 5 mg/L transferrin [Gibco], 10 μg/L somatostatin [Sigma, Deisenhofen, Germany], 3.625 μg/L hydrocortisone [Sigma], and 10 μg/L Gly-His-Lys [Sigma]), that is, 5H medium, or by the 5 hormones or growth factors plus 10 IU/L bovine TSH (Sigma), that is, 6H medium (*5*). Additionally, all media were enriched by 1% heat-inactivated fetal calf serum (batch 06Q2295F; Gibco) and by a premixed solution of antibiotics (5 mL/L streptomycin/penicillin; Gibco).

The number of cells suspended in medium was calculated after counting in a Neubauer hemocytometer chamber. Cells were seeded into tissue culture flasks (Greiner, Frickenhausen, Germany) with grounds of 75 cm^2. Media were added until the volume totaled 10 mL. Cultures from identical tissue samples were incubated in parallel by 5H medium and by 6H medium. The flasks were deposited with released caps in an incubator (Heraeus, Hanau, Germany) with a humidified atmosphere of 5% carbon dioxide in air at a temperature of 37°C.

At least 2 cultures were needed for direct comparison (5H medium vs. 6H medium). Whenever possible, we prepared 4 or even 6 cultures to perform duplicate or triplicate measurements. The number of cultures prepared was determined by the size of the tissue samples.

Storage of Cell Cultures

The cells were left in the incubator for at least 4 d to settle and adhere on the ground. Integrity of cell cultures was confirmed by morphologic criteria under an inverted microscope (Olympus America Inc., Melville, NY). Culture media had to be clear to indicate the absence of microbial contamination. Each time that media were renewed, samples from the used media were collected for measurement of Tg content. Before uptake was measured, culture media were changed several times to enable the cells to adapt to culture conditions. Each culture was consecutively incubated by media without TSH or media including TSH, exclusively.

Quantification of Tg

Concentrations of Tg in the culture media were measured by radioimmunoassay (DYNOtest Tg; Brahms, Hennigsdorf bei Berlin, Germany). The results were obtained as concentrations (ng/mL). Because the total volume of the media was constantly 10 mL, the concentration (ng/mL) multiplied by 10 (mL) revealed the net content (ng) of Tg in the media.

For quantification of Tg that had been liberated by thyrocytes, the total value had to be corrected for the amounts of Tg in fresh media, which were a fraction of the fetal calf serum. For this purpose, the Tg content in fresh media was also measured.

Consecutive measurements yielded accumulation curves of Tg as a function of time (Fig. 1). Accumulation curves displayed the total release of Tg as a function of time over the total incubation period. For correlative considerations of released Tg and intracellular uptake of ^{201}Tl, we exclusively regarded the amounts of Tg that had been secreted within the incubation periods before radioactive incubations. Because accumulation curves were found not to be linear, these quantities were supposed to reflect the secretory activity at the time of radioactive incubation more reliably than did the cumulative curves.

Liberation of Tg by cultured thyrocytes was normalized to 1 million cells and to 1 h of incubation to make the results comparable with each other despite differences in cell densities and incubation periods. The amounts were expressed as ng/h/million cells (Table 1).

Uptake Studies

Uptake measurements were preceded by incubation of cell cultures by ^{201}Tl (Amersham Biosciences, Piscataway, NJ) (Table 2). Activities (from 8.2 to 91 kBq) were added to fresh media. Uptake values were measured by a γ-counter (Caprac; Capintec Inc., Ramsey, NJ) that had an energy-level range of 15–100 keV.

One milliliter of each radioactive medium was measured as a reference (fraction I) to calculate the total activity to which a cell culture was exposed (fraction I × 10). Ten milliliters of fresh radioactive media were added to each cell culture after the used media had been removed. From the removed media, 2-mL samples were collected for quantification of Tg by radioimmunoassay.

Incubation was stopped after 1 h by removing the radioactive incubation media from the cell layers. Five milliliters of trypsin–ethylenediaminetetraacetic acid (Gibco) were added to the cell layers to induce their detachment from the plastic surfaces of the culture flasks. Flasks with trypsin were deposited in the incubator

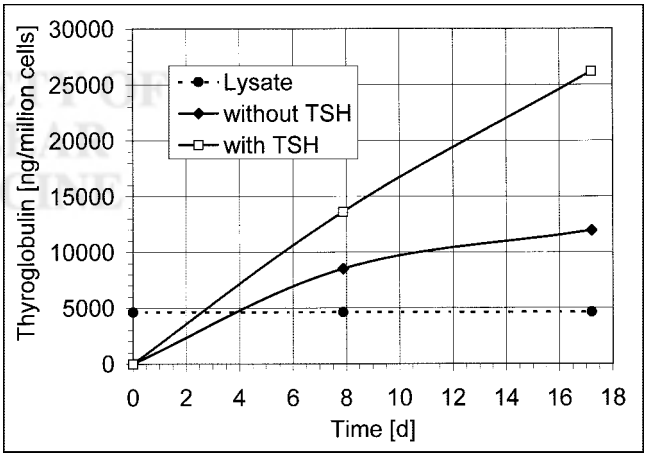

FIGURE 1. Liberation of Tg into culture media as function of time. Accumulation of Tg is markedly enhanced after stimulation by TSH. Curves are representative for primary cultures from human thyroid tissues. Dotted line marks amount of Tg measured in lysate of 1 million cells at time of inoculation. This culture was not included in set of cultures investigated for uptake of ^{201}Tl.

TABLE 1
Specifications of Cell Cultures: Release of Thyroglobulin

Tissue no.	Culture no.	TSH (IU/L)	No. of cells inoculated ($\times 10^6$)	Incubation period (h)	Amount of Tg in medium (ng)	Release of Tg (ng/h/million cells)
1	1-1	0	2.590	120	2,842.3	9.15
	1-2	10	2.590	120	7,622.7	24.53
2	2-1	0	2.590	120	1,761.3	5.67
	2-2	0	2.590	120	1,650.3	5.31
	2-3	0	2.590	120	1,553.3	5.00
	2-4	10	2.590	120	5,511.3	17.73
	2-5	10	2.590	120	3,662.3	11.78
	2-6	10	2.590	120	4,724.3	15.20
3	3-1	0	2.860	264	6,254.3	8.28
	3-2	0	2.860	264	5,998.3	7.94
	3-3	0	3.000	264	6,109.3	7.71
	3-4	10	2.860	264	8,369.3	11.08
	3-5	10	2.860	264	8,769.3	11.61
	3-6	10	3.000	264	8,238.3	10.40
4	4-1	0	1.965	316	2,570.3	4.14
	4-2	0	1.965	316	2,415.3	3.89
	4-3	10	1.965	316	7,425.3	11.96
	4-4	10	1.965	316	4,806.3	7.74
5	5-1	0	2.060	316	1,750.3	2.69
	5-2	10	2.060	249	2,355.3	3.62
6	6-1	0	2.000	249	1,645.3	3.30
	6-2	0	2.000	249	1,711.3	3.44
	6-3	10	2.000	249	4,658.3	9.35
	6-4	10	2.000	249	4,686.3	9.41
7	7-1	0	2.000	249	5,189.3	10.42
	7-2	0	2.000	249	4,586.3	9.21
	7-3	10	2.000	249	8,622.3	17.31
	7-4	10	2.000	249	8,642.3	17.35

for 5–8 min until detachment of all cells had been visually verified under the microscope. The enzyme was then inactivated by 6 mL fresh media without ^{201}Tl. From the suspension (total volume, 11 mL), exactly 10 mL were aspirated and pipetted into a 15-mL plastic tube. By centrifugation at 500*g*, a 10-mL supernatant consisting of trypsin and medium was separated from a pellet of cells.

The following steps were performed to precisely distinguish intracellular activity from activity in the surrounding media with a minimum of manipulations of the pellet. From the 10 mL of supernatant, exactly 9 mL were removed, leaving 1 mL of supernatant with the cell pellet in the tube. The activity in 1 mL of the removed supernatant was measured (fraction II) and subtracted from the value measured in the vessel containing the cell pellet plus 1 mL of supernatant (fraction III). This value (fraction III − fraction II), because it had been corrected for the activity in the liquid fraction surrounding the cells, was supposed to represent the amount of intracellular activity. Uptake values were obtained by calculating the ratios of intracellular uptake using the total activity that had been added to the cell culture (fraction I × 10).

After the γ-counting, the cell pellet was resuspended in 3 mL of medium and the cells were counted in a Neubauer hemocytometer chamber. Uptake values were normalized to 1 million cells and were finally expressed as %/million cells.

Aspecific adsorption of radioactivity on plastic materials has been reported to be considerable (*6*). Therefore, the activities of all fractions (media after 1 h of incubation, supernatant, and cellular uptake) were added and compared with the activities in fresh media before incubation. The resulting difference was supposed to reflect the share of activity that was adsorbed to the surfaces of laboratory materials.

Statistical Analysis

Data of the continuous variables "release of Tg" and "uptake of ^{201}Tl" were summarized descriptively using arithmetic means and SDs. Because the empiric distribution of these variables showed some left-skewing, data were logarithmically transformed before further statistical analysis.

The effect of the 2 culture conditions on these parameters was analyzed using a paired *t* test on the tissue-specific values averaged over the cultures. Because of this averaging, the variability of values within the cultures is not reflected in the analysis. Therefore, separate statistical models for Tg release and ^{201}Tl uptake were set up that acknowledge the way in which the replicate data within the cultures were collected and make full use of all available measurements. The generalized estimating equations (GEE) technique was used to analyze the effect of the 2 culture conditions within the corresponding generalized linear models. All probability values reported are 2-sided. The significance level for all analyses has been set at 5%; thus, $P < 0.05$ is considered statistically significant.

The correlation between ^{201}Tl uptake and Tg release is illustrated by a linear regression analysis and quantified by the Pearson product moment correlation calculated using the averaged tissue-specific values. The point estimate is accompanied by the 95% confidence interval to indicate the precision of the estimate. The

TABLE 2
Specifications of Cell Cultures: ^{201}Tl Uptake

Tissue no.	Culture no.	TSH (IU/L)	Activity (kBq)	Total activity in medium (kcts/min/10 mL)	Activity in cell pellet (kcts/min)	No. of cells in pellet (million cells)	Cellular uptake of ^{201}Tl (%/million cells)
1	1-1	0	91.0	5,064.00	7.300	1.230	0.117
	1-2	10	91.0	5,361.00	18.670	1.050	0.332
2	2-1	0	8.2	267.30	0.165	0.273	0.227
	2-2	0	8.2	256.50	0.045	0.311	0.056
	2-3	0	8.2	269.55	0.081	0.269	0.112
	2-4	10	8.2	254.61	0.030	0.291	0.041
	2-5	10	8.2	257.04	0.189	0.304	0.243
	2-6	10	8.2	267.75	0.664	0.296	0.839
3	3-1	0	75.0	2,969.00	1.095	0.297	0.124
	3-2	0	75.0	2,969.00	1.062	0.228	0.157
	3-3	0	75.0	2,969.00	4.210	0.331	0.429
	3-4	10	75.0	2,741.00	4.910	0.186	0.962
	3-5	10	75.0	2,741.00	4.154	0.169	0.894
	3-6	10	75.0	2,741.00	4.898	0.239	0.748
4	4-1	0	25.0	1,269.00	0.354	0.161	0.173
	4-2	0	25.0	1,269.00	0.975	0.236	0.326
	4-3	10	25.0	1,326.00	1.468	0.160	0.692
	4-4	10	25.0	1,326.00	3.752	0.192	1.472
5	5-1	0	25.0	1,269.00	1.646	0.287	0.452
	5-2	10	25.0	1,326.00	4.686	0.306	1.157
6	6-1	0	44.0	1,411.20	1.574	0.149	0.749
	6-2	0	44.0	1,411.20	0.206	0.171	0.085
	6-3	10	44.0	1,292.40	1.399	0.091	1.188
	6-4	10	44.0	1,292.40	0.504	0.164	0.237
7	7-1	0	44.0	1,411.20	0.562	0.327	0.122
	7-2	0	44.0	1,411.20	0.513	0.229	0.159
	7-3	10	44.0	1,292.40	1.318	0.193	0.527
	7-4	10	44.0	1,292.40	1.069	0.173	0.477

statistical computations were performed using the SAS software package (version 8.1; SAS Institute, Cary, NC).

RESULTS

Primary cell cultures from human thyroid tissue were viable for several weeks. The cells were accumulating ^{201}Tl and releasing Tg. Furthermore, our results clearly indicate that liberation of Tg and uptake of ^{201}Tl were enhanced by TSH (Fig. 1). Cultures in the absence of TSH secreted 6.049 ± 2.905 ng Tg/h/million cells. In contrast, cultures derived from identical tissue samples produced 12.949 ± 6.711 ng Tg/h/million cells in the presence of 10 IU/L bovine TSH (Fig. 2). This doubling of Tg release in the presence of 10 IU/L bovine TSH reached statistical significance in the standard analysis of the averaged values (*t* test, $P = 0.001$) and in the more sophisticated analysis of all available data (GEE analysis, $P < 0.0001$).

Uptake of ^{201}Tl was 0.249% ± 0.137%/million cells in cultures without TSH and 0.718 ± 0.332%/million cells in corresponding cultures exposed to TSH (Fig. 3). The difference in the uptake of ^{201}Tl between the 2 culture conditions was statistically significant using both analytic approaches (*t* test, $P = 0.0002$; GEE analysis, $P = 0.0004$).

Aspecific adsorption of radioactivity was 5.06% ± 2.65% ($n = 28$). Thus, the share of activity that was adsorbed by the surfaces of laboratory materials was regarded as a constant minor quantity with little potential to falsify the results.

Release of Tg and uptake of ^{201}Tl correlate inversely; that is, the comparison of individual cultures reveals that cells secreting higher amounts of Tg tend to accumulate less ^{201}Tl than do cultures releasing low amounts of Tg, and vice versa (Fig. 4). The Pearson correlation coefficients were −0.7895 (95% confidence limits, −1 and −0.5367) for cells cultured without TSH and −0.7156 (95% confidence limits, −0.9099 and −0.5213) for cultures stimulated by TSH.

DISCUSSION

In this study, we examined the uptake of ^{201}Tl into primary thyroid cell cultures. Primary cell cultures are a well-established model of the functioning, TSH-sensitive human thyroid. In particular, these cell cultures have been shown to produce and secrete Tg, and this process has been shown to be TSH dependent (*7*). These findings fully agree with our data.

Because Tg is a specific product of differentiated thyrocytes, accumulation of Tg in the culture media proves that functionally active thyrocytes are present within the heterogeneous cell populations of which primary cultures consist. By comparison with the Tg content found in the lysates

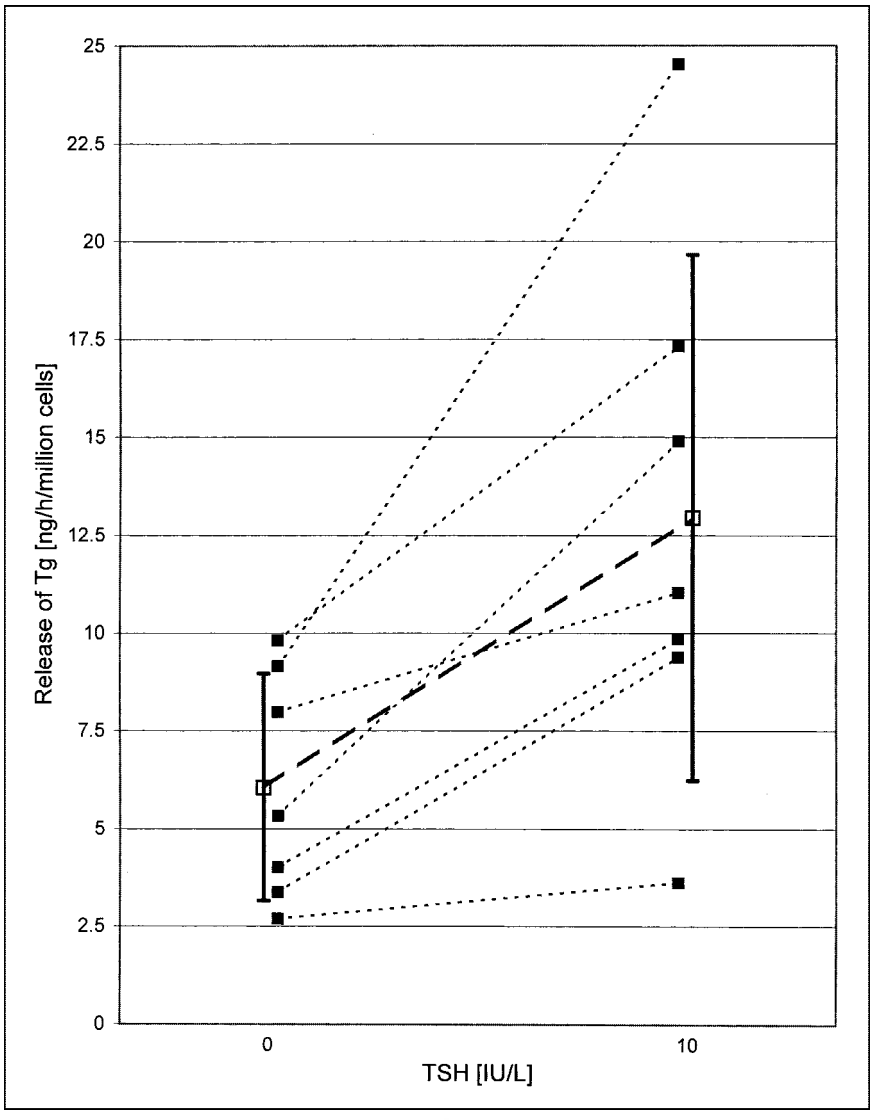

FIGURE 2. Effect of TSH on differentiated functions of human thyrocytes. Release of Tg by primary cultures is enhanced in presence of 10 IU/L bovine TSH. Error bars indicate SDs of average values.

of cells at the time of inoculation, we could show that the amounts accumulating in the culture media were exceeding this value, thus indicating active secretion during cell culture (Fig. 1).

The principal finding of our study is that ^{201}Tl uptake into these cultures depends significantly on TSH. To the best of our knowledge, ^{201}Tl uptake into primary cell cultures from thyroid tissue has not been studied; however, numerous studies are investigating the effect of TSH stimulation on the metabolism of thyrocytes. In particular, TSH in thyroid cells has been noted to display ubiquitous effects on various aspects of thyroid function (*8*). Because ^{201}Tl is transported into the cell through the action of Na^+/K^+-adenosine triphosphatase (ATPase) (*9*), it seems plausible that the observed increase in ^{201}Tl uptake after stimulation by TSH reflects an enhanced activity of this enzyme. Indeed, Na^+/K^+-ATPase is reported to be stimulated by proteinkinase A, which is activated by cyclic adenosine monophosphate, an intracellular messenger that is, in turn, controlled by TSH (*10–12*).

Measurements on cultures derived from identical tissue samples exhibited a surprisingly large variability in ^{201}Tl uptake, although all cell cultures had been exposed to the same environmental conditions. This variability was probably caused by the manipulations on the cells after incubation—for example, by the action of trypsin, which had to be added to promote detachment of the cells from the bottoms of the culture flasks. The trypsin may have damaged the cells to different degrees, thus causing variable release of ^{201}Tl. Furthermore, efflux of ^{201}Tl after incubation may also have contributed to the variability of the data. The idea that variabilities are caused by proceedings at the end of the incubation is supported by the absence of comparably large variations when the secretion of Tg during long-term culture is considered.

To deal with the variability of the data, we additionally analyzed the data in a statistical modeling framework We set up generalized linear models for the logarithmically transformed Tg release and ^{201}Tl uptake, acknowledging the way in which the replicate data within the cultures were

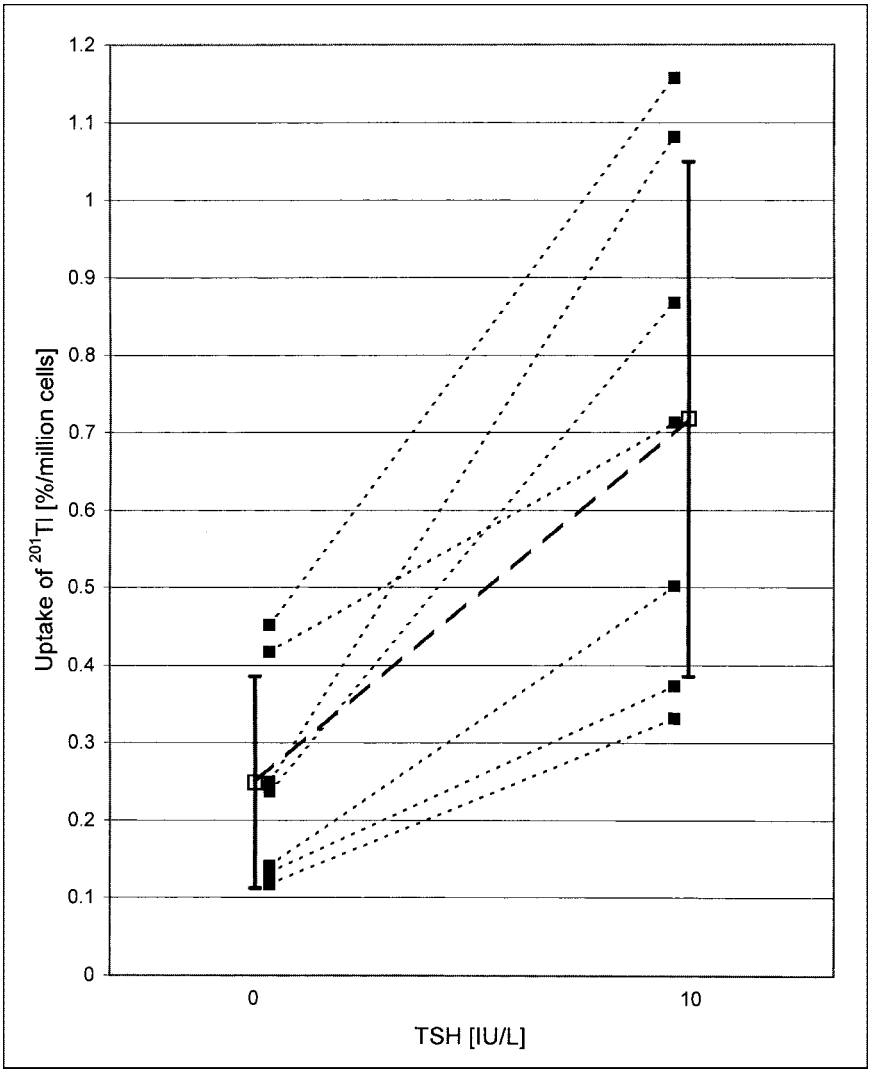

FIGURE 3. Effect of TSH on differentiated functions of human thyrocytes. Uptake of ^{201}Tl by primary cultures is enhanced in presence of 10 IU/L bovine TSH. Error bars indicate SDs of average values.

collected. The GEE technique was then used to analyze the effect of 2 culture conditions on Tg release and ^{201}Tl uptake. Although the data were highly variable in some cultures, the GEE analysis corroborated the significant effect of the culture conditions on these parameters.

Data obtained in animal models support our observation of the dependence of ^{201}Tl uptake on TSH in human primary thyroid cell cultures. Maayan et al. (*8*) characterized ^{201}Tl kinetics in the thyroid glands of mice after having challenged the uptake by different metabolic interventions addressing the action of TSH. Responsiveness of ^{201}Tl uptake was proven by enhancement after TSH stimulation induced by propylthiouracil, whereas ^{201}Tl uptake was suppressed under a diet containing thyroxine with TSH-suppressive doses. The authors concluded that, like ^{131}I, ^{201}Tl accumulates in response to TSH stimulation.

A similar study was conducted by Oster et al. (*13*). These authors measured thyroid ^{201}Tl uptake in rats receiving intraperitoneal TSH injections, rats receiving intraperitoneal injections of triiodothyronine, and rats receiving intravenous perchlorate and compared the results with those obtained from untreated control animals. The ^{201}Tl concentration was significantly increased in TSH-treated animals and was decreased in triiodothyronine-treated animals when compared with untreated control animals. Treatment by perchlorate did not lead to significant alterations. Experimental evidence of the TSH dependence of ^{201}Tl uptake in human thyroid glands was missing; however, the accumulation of ^{201}Tl was reduced in the thyroid glands of patients treated with amiodarone. Because this was observed in both euthyroid and hyperthyroid patients, inhibition of ^{201}Tl uptake was concluded to be caused by the inhibitory effect of iodides on adenyl cyclase and its stimulation by TSH (*14*).

Basically, the distribution of thallium is analogous to that of potassium in all tissues (*15*). The tumor-seeking properties of ^{201}Tl scintigraphy are related to the increased potassium content of malignant tissues (*15,16*). Concordantly, in various tumor cells and also in malignant thyroid nodules, active transport by Na^+/K^+-ATPase has been found to be accelerated (*17,18*).

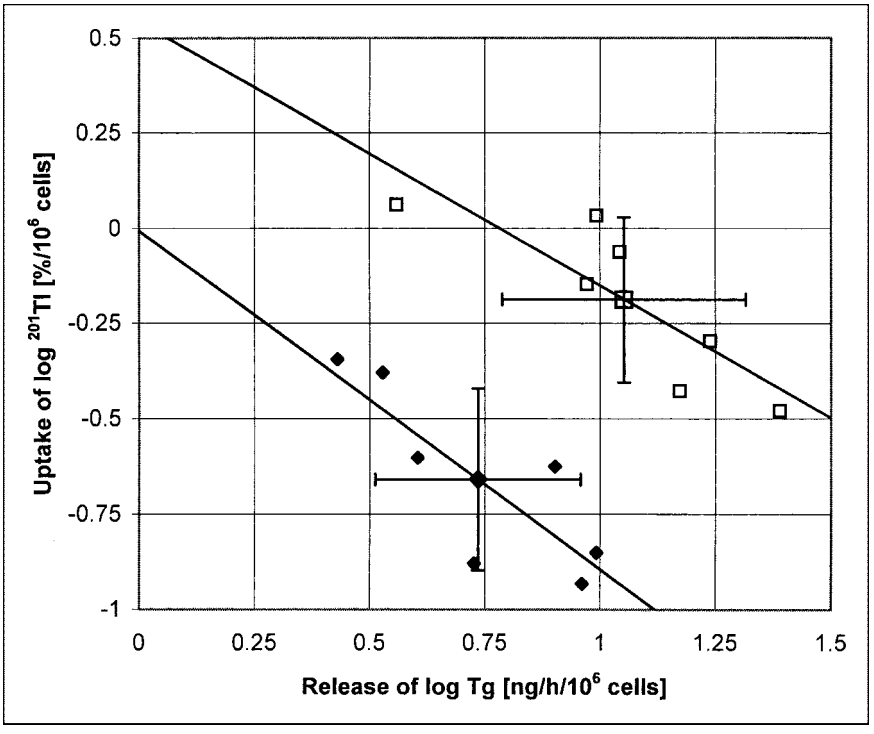

FIGURE 4. Correlation of ^{201}Tl uptake (%/million cells) with release of Tg (ng/h/million cells). Linear regressions for logarithmically transformed parameters illustrate inverse relationships between parameters in cultures grown in absence of TSH (◆) and in cultures stimulated by TSH (□). Error bars indicate SDs of average values.

Uptake of ^{201}Tl has been found to reflect the proliferative activity of thyroid nodules (*19*). Consecutively, in dedifferentiated tumors, uptake of ^{201}Tl may be higher than in differentiated ones. This would make scintigraphy with ^{201}Tl a valuable tool, especially for the detection of dedifferentiated tissues originating from the thyroid gland.

The amount of Tg synthesis is supposed to indicate differentiation, because undifferentiated thyroid tissues fail to produce this protein. In such tissues, an elevated uptake of several radiopharmaceuticals, including ^{201}Tl, has been reported in vivo (*20–22*). Interestingly, we observed an analogous inverse relationship between ^{201}Tl uptake and the liberation of Tg in our cell cultures, both in the presence and in the absence of TSH. This observation could have been caused by an incipient dedifferentiation of a part of our cell cultures, although cellular features of this process could not be documented in our model.

Scintigraphy with ^{201}Tl is a routine examination for detecting metastases of differentiated thyroid carcinoma (*3,23,24*). However, the reported sensitivity and specificity vary widely (*25–27*). Nevertheless, by general agreement, ^{201}Tl scintigraphy can be used as an additional diagnostic device for the follow-up of patients with suspected or proven thyroid metastases that do not trap iodine (*3,26,27*).

TSH is generally considered not to be a major determinant of ^{201}Tl uptake. Contrary to ^{131}I scintigraphy, which is routinely performed after withdrawal of hormone in the hypothyroid state, ^{201}Tl scintigraphy may also be performed while the patient is still receiving thyrosuppressive medication, as has been shown by several in vivo studies revealing ^{201}Tl uptake in thyroid tissues at suppressed levels of TSH (*25,27–31*).

Application during ongoing thyroid hormone replacement is a considerable advantage of ^{201}Tl over ^{131}I. This advantage becomes evident when one considers the discomfort of patients and the potential risk of promoting proliferation through secretion of TSH that are associated with the withdrawal of thyroid hormone medication. Our data, however, suggest that the sensitivity of ^{201}Tl scintigraphy may be improved if it is performed on hypothyroid patients, at least when iodine-negative metastases of thyroid carcinomas that still respond to TSH are studied. Such metastases are present in most cases of well-differentiated thyroid carcinoma even when failure of iodine uptake has occurred, as illustrated by the fact that the production and secretion of Tg by these thyroid carcinomas is still TSH dependent as evidenced in the hypothyroid state. Sensitivity to TSH is also a feature of our primary cell cultures. One may therefore hypothesize that the sensitivity of ^{201}Tl scintigraphy parallels that of the measurement of Tg (*32*), because both uptake of ^{201}Tl and liberation of Tg were increased by TSH in our cell cultures. Our data encourage studies on patients and on cell cultures from thyroid carcinomas to prove this hypothesis.

Although ^{201}Tl is widely used in the diagnostic work-up of patients with thyroid carcinoma, ^{18}F-FDG and also ^{99m}Tc-methoxyisobutylisonitrile and ^{99m}Tc-tetrofosmin have recently gained more attention because these tracers offer distinct physical advantages to imaging (*20,33–38*). Interestingly, recent in vivo studies have shown that, in metastases of well-differentiated thyroid carcinomas, uptake of FDG also is TSH dependent (*39,40*). It would therefore be interesting to study this relationship in our in vitro model as well.

CONCLUSION

Our in vitro data suggest that withdrawal of thyroid hormone substitution has the potential to improve the sensitivity of ^{201}Tl scintigraphy for detecting thyroid remnants or recurrences. Further studies are needed to confirm these findings on thyroid carcinoma cell cultures. Clinical studies should address the extent to which the responsiveness of ^{201}Tl uptake to TSH is clinically relevant.

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