In Vitro Studies on the Signal Transduction of Thyroidal Uptake of ^{18}F-FDG and ^{131}I-Iodide

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Glucose metabolism in radioiodine-negative metastases of differentiated thyroid carcinomas (DTC) may still be increased by thyroid-stimulating hormone (TSH) as demonstrated by PET with ^{18}F-FDG. The mechanisms of signal transduction involved in that process are as yet not completely understood. Therefore, the aim of this study was to investigate the effects of TSH, of an analog of cyclic adenosine monophosphate (dibutyryl cyclic AMP $(Bu)_2$cAMP), and of inhibitors of the phosphatidylinositol 3-kinase (PI3-kinase) and of the protein kinase A (PKA) on ^{18}F-FDG and radioiodide uptake in the thyroid cell line FRTL-5. **Methods:** FRTL-5 cells were cultured in the presence of hormones with or without 1 mU/ mL TSH. Glucose carrier (GLUT-1) was determined by Western blot analysis. Cells were incubated with 0.5–1.0 MBq/mL ^{18}F-FDG for 1 h or 18–37 kBq/mL ^{131}I for 45 min, respectively, and tracer uptake was related to protein concentration. $(Bu)_2$cAMP (1 mmol/L) was used as cAMP enhancer, H89 (0.25–25 μmol/L) as selective PKA inhibitor, and wortmannin (1 μmol/L) as inhibitor of PI3-kinase. **Results:** TSH induced a 2.6-fold ± 0.5 increase of radioiodide uptake in FRTL-5 cells ($P < 0.001$, $n = 8$). The use of wortmannin inhibited TSH-induced uptake of ^{131}I only moderately by 21.1% ± 3.5% ($P < 0.05$, $n = 8$), whereas H89 markedly blocked the effect of TSH by 53.8% ± 16.7% ($P < 0.001$, $n = 8$). TSH enhanced GLUT-1 concentration of FRTL-5 cell membrane preparations by a factor of 1.6 ($n = 3$). TSH-treated cells showed a 2.6-fold increased uptake of ^{18}F-FDG ($P < 0.001$, $n = 20$). Stimulation by $(Bu)_2$cAMP analogously increased ^{18}F-FDG uptake ($P < 0.001$, $n = 20$). Wortmannin, but not H89, significantly inhibited TSH- and $(Bu)_2$cAMP-stimulation of ^{18}F-FDG uptake by 42% ± 25% ($P < 0.001$, $n = 20$) and 42% ± 31% ($P < 0.001$, $n = 20$), respectively. **Conclusion:** The effect of TSH and cAMP on ^{18}F-FDG uptake by FRTL-5 cells is mediated by PI3-kinase and not by PKA, thus differing from the mechanism of radioiodide accumulation of this cell line. This observation is one possible explanation for the persistence of TSH-dependent ^{18}F-FDG uptake in radioiodine-negative metastases of DTC.

Key Words: ^{18}F-FDG; thyroid; FRTL-5 cells; thyroid-stimulating hormone; PET

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Over the past decade, PET using ^{18}F-FDG has gained widespread acceptance as a diagnostic tool in oncology (*1*). This has motivated research into factors governing cellular ^{18}F-FDG accumulation occurring via action of the glucose carriers and hexokinase. In neoplastic cells, an upregulation of the glucose transport protein GLUT-1 has been shown repeatedly to account for increased tracer uptake (*2–6*).

Furthermore, it has become clear that this variable is dependent on the action of several regulatory substances differing, however, between the cell types investigated: In tumor cells, hypoxia-inducible factor 1 exerts a major influence (*2*), cytokines govern ^{18}F-FDG uptake in inflammatory cells (*7,8*), vascular endothelial growth factor increases this variable in endothelial cells (*9*), and—last, but not least—thyroid-stimulating hormone (TSH) considerably increases ^{18}F-FDG accumulation in differentiated thyroid cells in vitro (*10*).

The latter has also been demonstrated in vivo for metastases of differentiated thyroid cancer (DTC), obviously still expressing the TSH receptor (*11*). The clinical consequence of this finding is that ^{18}F-FDG PET aimed at detecting and localizing radioiodine-negative metastases of this tumor is usually performed under TSH stimulation (*12–16*). This can be achieved by withdrawal of L-thyroxine in the athyroid patient, leading to considerable discomfort. Intramuscular injection of recombinant human TSH is an alternative to this procedure but is quite expensive.

Dedifferentiation of thyroid cancer tissue may lead to the loss of its capacity to accumulate iodide. This process is often accompanied by an increase in glucose metabolism. The intracellular factors responsible for this so-called "flip–flop" phenomenon are largely unknown. A further elucidation of the regulation of iodide and glucose metabolism in normal and neoplastic thyroid tissue will also help to understand the clinical behavior of thyroid cancer cells and hopefully allow the development of new diagnostic and therapeutic strategies.

Therefore, the aim of this study was to investigate the effect of TSH, of an analog of cyclic adenosine-monophosphate (dibutyryl cyclic AMP $(Bu)_2$cAMP), and of inhibitors of the phosphatidylinositol 3-kinase (PI3-kinase) and of the protein kinase A (PKA) on ^{18}F-FDG and radioiodide uptake in the thyroid cell line FRTL-5. cAMP, PI3-kinase, and PKA

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are all classic intracellular messengers potentially involved in the signal transduction of the interaction of a first messenger with the extracellular binding domain of a membrane receptor.

MATERIALS AND METHODS

Reagents

$Na^{131}I$ (product code IBSSO) was obtained from GE Healthcare Bio-Sciences (Amersham Buchler GmbH). ^{18}F-FDG was purchased from PET Net GmbH. $(Bu)_2$cAMP, wortmannin (from *Penicillium funiculosum*), *N*-[2-(*p*-bromocinnamyl-amino)ethyl]-5-isoquinolinesulfonamide (H89), bovine TSH, and Coon's modified Ham's F-12 medium were supplied by Sigma-Aldrich. Fetal calf serum (FCS), phosphate-buffered saline (PBS), and trypsin/EDTA (EDTA is ethylenediaminetetraacetic acid) were obtained from Invitrogen/Gibco.

Cell Culture

The rat thyroid cell line FRTL-5 was obtained from the European Collection of Cell Cultures (no. 91030711) and grown in a culture medium based on Coon's F-12 solution (Sigma) supplemented with a 6-hormone mixture (10 mg/L insulin, 5 mg/L transferrin, 10 μg/L somatostatin, 10 nmol/L hydrocortisone, 10 μg/L glycyl-L-histidyl-L-lysine acetate, and 1 U/L bovine TSH [Sigma]; 6H medium) (*17*). The 6H medium was supplemented with 5% FCS and used for FRTL-5 cell culture in a humidified incubator at an atmosphere of 5% CO_2 at 37°C. FRTL-5 cells were routinely subcultured every 3–4 d. The cell viability of FRTL-5 cells was proven by trypan blue staining.

Immunocytochemical TSH Receptor Staining

FRTL-5 cells were cultured in 6H medium as described. TSH-starved cells were obtained by changing the medium to 5H medium (6H medium without TSH) after reaching 70%–80% confluence in a 75-cm^2 cell culture flask. TSH deprivation was continued for 4 d. Approximately 100,000 FRTL-5 cells in 6H medium or 200,000 FRTL-5 cells in 5H medium were seeded in 8-well chamber slides (Nunc). After 24 h the cell layers were 50%–60% confluent and immunocytochemical staining was performed by the alkaline phosphatase–antialkaline phosphatase technique (APAAP kit; Dako) according to the specifications of the manufacturer. Briefly, cells were chilled on ice, washed with Tris-buffered saline (TBS, pH 7.6), and fixed in cold methanol for 1.5 min. After washing for 5 min in TBS, cells were incubated with the TSH receptor antibody-1 (ready-to-use solution, TSHR mouse monoclonal antibody, clone 4C1/E1/G8; Lab Vision Corp.) as primary antibody for 30 min at room temperature. Slides were washed (TBS, 5 min) and a rabbit immunoglobulin fraction to mouse immunoglobulins (Dako) was applied as secondary antibody for 30 min at room temperature. After washing, slides were exposed to the alkaline phosphatase–antialkaline phosphatase immune complex, followed by washing for 5 min in TBS and repeating incubation with the secondary antibody and the APAAP complex for 10 min, respectively. Finally, cells were exposed to the substrate solution (Tris buffer containing naphthol phosphate, Fast Red, and levamisole, pH 8.2; Dako) for 2 × 7 min. Slides were briefly dipped in water and treated with AquaTex (Merck) to conserve staining results. Negative control slides were obtained by omitting the primary antibody. Cells were examined at ×20 using an Olympus light microscope.

Crude Membrane Preparation

Total cellular membranes were isolated from FRTL-5 cells cultured in 5H medium for 72 h and FRTL-5 cells cultured in 6H medium (control). Briefly, the media of control and TSH-deprived cultures were aspirated, and the cultures were washed with cold PBS. PBS containing 0.04% EDTA was added to each culture, and the cells were scraped and transferred to a 15-mL tube. The cells were centrifuged at 1,000 rpm (200*g*) for 10 min at room temperature. The supernatant was aspirated and the cell pellets were resuspended in 1.0 mL radioimmunoprecipitation assay (RIPA) buffer (0.25 mol/L NaCl, 0.05 mol/L 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid [HEPES], 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 0.5% sodium dodecyl sulfate–containing protease inhibitors [1:100; Sigma P8340]) at 4°C and incubated for 30 min (4°C). Subsequently, the resulting suspension was centrifuged at 3,500 rpm (1,200*g*) at 4°C for 10 min. The total cellular membranes in the resultant supernatant were collected by centrifugation at 100,000*g* for 60 min at 4°C and resuspended in 50 μL solubilization buffer (PBS containing 0.1% Triton X-100). An aliquot (typically 2 μL) of this solution was diluted with 0.9% NaCl solution (1:200) for measuring protein concentration by the QuantiPro bicinchoninic acid (BCA) assay kit (Sigma) using bovine serum albumin (BSA) as a standard.

Western Blot Analysis of GLUT-1

Electrophoresis of membrane preparations was performed on aliquots of each sample on a 10% polyacrylamide gel (precast NuPAGE Novex Bis-Tris gel; Invitrogen) and transferred to nitrocellulose membranes by using a Hoefer miniVE blot module (Amersham Biosciences). For direct comparison of samples, an aliquot of each sample corresponding to 3.7 μg (determined by BCA assay using BSA as standard) was loaded on the gel. An aliquot of 2.5 μL of rat glucose transporter type 1 (PC-Glut-1; FabGennix Inc.) was loaded on each gel as a positive control and for comparison of molecular mass. SeeBlue prestained standard (Invitrogen) was loaded on each gel to allow visual control of the polyacrylamide gel and blotting results. Nitrocellulose membranes were incubated in blocking solution (5% nonfat dry powdered milk in TBS buffer containing 50 mmol/L Tris and 120 mmol/L NaCl, pH 7.5) for 1 h at room temperature. Western blot analysis was performed by incubating membranes with a rabbit polyclonal antihuman glucose transporter-1 antibody (FabGennix Inc.) at a concentration of 1:1,000 overnight at 4°C. After washing in TBS buffer (0.1% Tween-20) for 3 × 15 min, the blots were incubated with an antirabbit secondary antibody coupled to horseradish peroxidase at a concentration of 1:1,000 in TBS buffer for 1 h at room temperature. After repeated washing (3 × 15 min, room temperature) in TBS buffer (0.1% Tween-20), development of the blots was performed with a chemoluminescence reagent (ECL Plus; Amersham Biosciences) according to the manufacturer's instructions. Blots were visualized by a Fluor-S MultiImager (Bio-Rad Laboratories), and the blot images were scanned and quantified using the software Quantity One (version 4.3.0; Bio-Rad Laboratories).

Determination of Intracellular ^{131}I Uptake

For ^{131}I uptake experiments, 10^5 cells per milliliter in 6H medium were seeded in 12-multiwell culture plates and the medium was changed to 5H medium (6H medium without TSH) after 24 h. TSH deprivation was continued for 4 d. Subsequently, FRTL-5 cells were either treated with 1 mU/mL TSH or 1 mmol/L $(Bu)_2$cAMP for 48 h in the presence and absence of 10 μmol/L

H89 (selective inhibitor of PKA) and 1 μmol/L wortmannin (irreversible inhibitor of PI3-kinase). The medium was discarded and cells were washed in Hanks' buffered saline solution (HBSS) containing 5.55 mmol/L glucose and 10 mmol/L HEPES. One milliliter of incubation buffer (HBSS/HEPES containing 10 μmol/L NaI) was added and incubated for 10 min. Five microliters (18–37 kBq) of $Na^{131}I$ in HBSS/HEPES were added to each well and incubation was continued for 45 min at 37°C. The incubation was terminated by cooling the cell layer with ice, and an aliquot from the incubation medium (50 μL) was withdrawn for radioactivity measurements. Each well was rapidly rinsed once with 1.0 mL cold HBSS/HEPES, and 0.5 mL of 0.1 mol/L NaOH solution was added to each well to dissolve the cells. The resulting cell suspension was transferred to tubes and used for radioactivity measurements (Wallac Wizard; Perkin Elmer). After homogenization, determination of protein concentration was performed by the method of Bradford (*18*). ^{131}I uptake was expressed as the percentage of whole ^{131}I radioactivity divided by the total protein mass (%/mg).

^{18}F-FDG Uptake Experiments

Before ^{18}F-FDG uptake experiments, TSH-deprivation was performed by seeding 1.5×10^5 cells/mL in 6H medium and changing to a deprivation medium (Coon's F12 medium containing 5 mg/L transferrin, 0.5% FCS) after 24 h according to the procedure described by Samih et al. (*19*). FRTL-5 cells were cultured in the deprivation medium for 24 h. Subsequently, the cells were either treated with 1 mU/mL TSH or 1 mmol/L $(Bu)_2$cAMP for 24 h (positive control) or maintained in the deprivation medium in the quiescent phase for further 24 h (negative control). The effect of H89 (selective inhibitor of protein kinase A [PKA]) in varied concentrations (0.25 μmol/L, 2.5 μmol/L and 25 μmol/L) and 1 μmol/L wortmannin (irreversible inhibitor of PI3 kinase) on TSH- and cAMP-induced ^{18}F-FDG uptake was studied by adding the inhibitor to the incubation medium 30 min before stimulation. ^{18}F-FDG (0.5–1 MBq, 10 μL) was added to each culture well containing a total volume of 1.0 mL and incubation was continued for 1 h at 37°C. An aliquot from the incubation medium (50 μL) was withdrawn and used for radioactivity measurements. The incubation was terminated by aspirating the medium rapidly and rinsing the cell layer with cold PBS. 0.5 mL of 0.1 mol/L NaOH solution was added to dissolve the cells. Radioactivity and protein concentration measurements were performed as described for radioiodide uptake experiments. ^{18}F-FDG uptake was expressed as percent of whole ^{18}F-FDG radioactivity divided by total protein mass (%/mg).

Coefficient of Variation of Tracer Uptake Experiments and Statistics

Statistical analysis was performed using SPSS software (version 11.0.1; SPSS Software GmbH). All data are expressed as mean ± SD. The coefficient of variation (COV) of ^{18}F-FDG uptake between and within assays was calculated as SD/mean × 100. ^{18}F-FDG uptake varied between experimental assays with a COV of 43.7% but showed less within-assay variation, demonstrated by a COV of 18.5% ± 4.9% as determined from 5 independent experiments, each performed in quadruplicate. Therefore, in the inhibition experiments with ^{18}F-FDG, the control values were set to 100% to allow for pooling of data from individual experiments. The same strategy was applied to the inhibition experiments with radioiodide (COV data not shown). All statistical results are based on the nonparametric Mann–Whitney test. The number of samples (n) used for statistical analysis and the number of independent experiments are given in the figure legends. P values < 0.05 were considered to be significant.

RESULTS

As shown in Figure 1, TSH-activated FRTL-5 cells cultured in the presence of a mixture of 6 hormones (6H medium) and TSH-starved cells that were cultured without TSH in 5H medium clearly expressed the TSH receptor.

TSH induced a 2.6-fold ± 0.5 increase of radioiodide uptake in FRTL-5 cells (163% ± 30%/mg, $P < 0.001$, $n = 8$) (Fig. 2), which was mimicked by using $(Bu)_2$cAMP (107% ± 6%/mg, $n = 4$, data not shown). The use of wortmannin inhibited TSH-induced ^{131}I only moderately (21.1% ± 3.5%, $n = 8$), whereas H89 markedly blocked the effect of TSH by 53.8% ± 16.7% ($P < 0.001$, $n = 8$) (Fig. 2)—that is, to a value comparable to that found in TSH-starved cells ($P > 0.05$, $n = 8$). Contrary to wortmannin, H89 also inhibited radioiodide uptake in TSH-starved cells (Fig. 2).

To determine the effect of TSH on GLUT-1 expression, membrane preparations of TSH-treated and untreated FRTL-5 cells were investigated by Western blot analysis (Fig. 3). The ratio of GLUT-1 concentration between TSH-stimulated and TSH-deprived cells in membrane preparations of equal whole protein mass was determined to be 1.60 ± 0.07 ($n = 3$, $P < 0.01$), indicating a significant reduction of GLUT-1 concentration in membrane preparations of TSH-deprived cells.

^{18}F-FDG uptake in the rat thyroid cell line FRTL-5 revealed a significant dependency on TSH and also on the cAMP enhancer $(Bu)_2$cAMP (Fig. 4). In comparison with

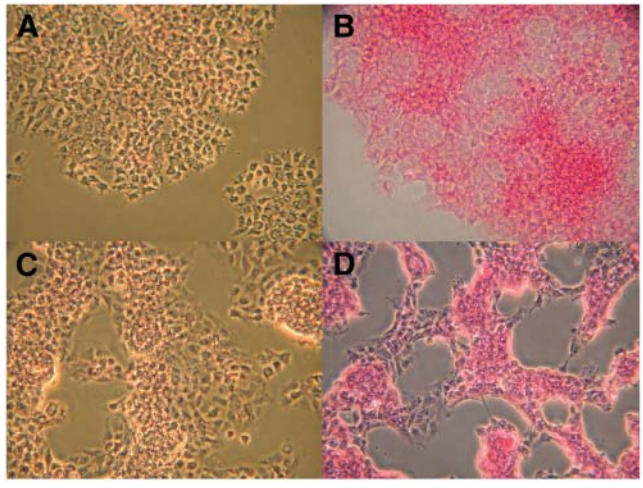

FIGURE 1. Immunocytochemical staining of TSHR on FRTL-5 cells. A mouse monoclonal TSHR antibody was used as primary antibody. TSH-treated FRTL-5 cells: (A) Negative control. (B) Primary antibody. TSH-starved FRTL-5: (C) Negative control. (D) Primary antibody.

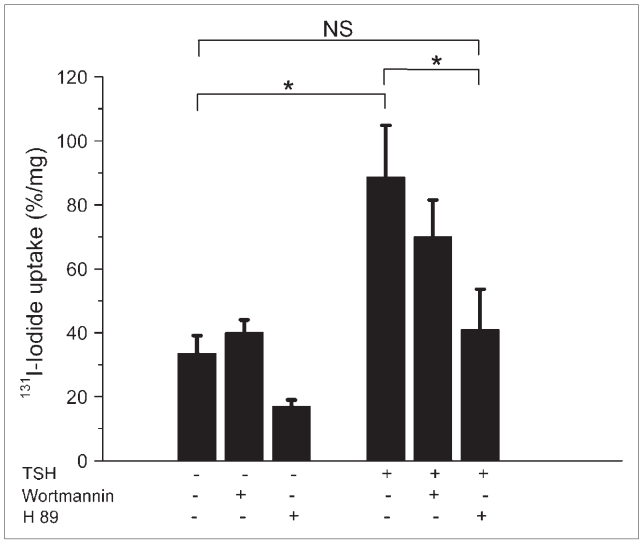

FIGURE 2. Uptake of ^{131}I in TSH-treated FRTL-5 thyroid cells. H89 significantly blocked TSH-induced uptake of ^{131}I by 53.8% ± 16.7% (*$P < 0.001$, $n = 8$) confirming PKA-mediated increase of radioiodide transport by TSH. Values are mean ± SD of 2 independent experiments, each performed in quadruplicate. NS = not significant ($P > 0.05$).

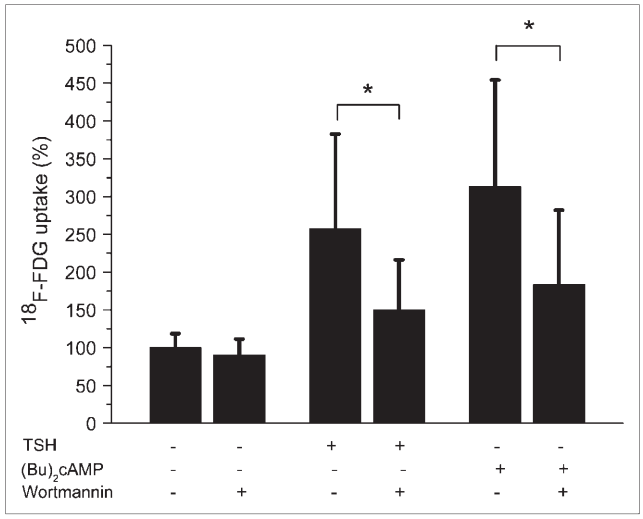

FIGURE 4. Inhibition of $(Bu)_2$cAMP- and TSH-induced increase of ^{18}F-FDG uptake in FRTL-5 cells by the PI3-kinase inhibitor wortmannin. Differences in ^{18}F-FDG uptake values between TSH-activated FRTL-5 cells (+TSH) and TSH-treated cells in the presence of the selective PI3-kinase inhibitor wortmannin (1 μmol/L) were statistically significant (*$P < 0.001$, $n = 20$). Values are mean ± SD of 5 independent experiments, each performed in quadruplicate.

untreated FRTL-5 cells, TSH-treated cells showed a 2.6-fold increased uptake of ^{18}F-FDG (257% ± 125% vs. 100% ± 19%, $n = 20$, $P < 0.001$). A similar effect on ^{18}F-FDG uptake was observed by treatment of the cells with $(Bu)_2$cAMP for 24 h (Fig. 4). As also shown in Figure 4, wortmannin, an irreversible inhibitor of PI3-kinase, significantly inhibited TSH- and cAMP-induced ^{18}F-FDG uptake by 42% ± 25% ($P < 0.001$, $n = 20$) and 42% ± 31% ($P < 0.001$, $n = 20$), respectively.

The selective cAMP-activated PKA inhibitor H89, an isoquinoline derivative, did not significantly influence ^{18}F-FDG uptake in FRTL-5 cells at concentrations between 0.25 and 25 μmol/L ($P > 0.05$, $n = 4$) (Fig. 5).

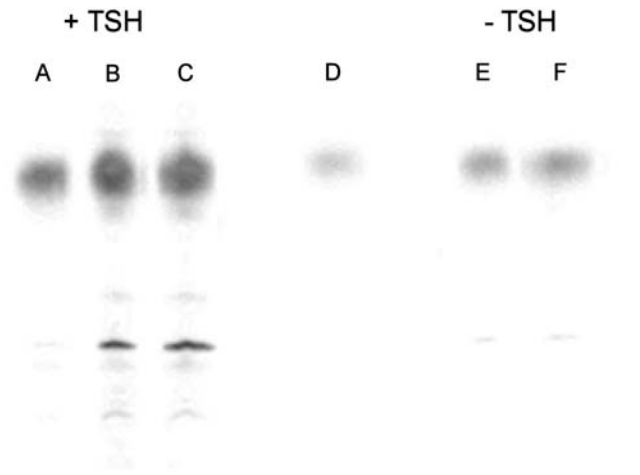

FIGURE 3. Representative Western blot analysis of GLUT-1 expression in TSH-treated and untreated FRTL-5 rat thyroid cells. Cells were cultured in absence of TSH for 3 d, and TSH (1 mU/mL) was added subsequently. Membrane preparations were obtained as described. Lines A, B, and C show expression of GLUT-1 in membranes of TSH-treated cells (different protein concentrations were loaded on the gel: A, 3.7 μg; B, 7.4 μg; C, 11 μg). Line D shows rat GLUT-1–positive control (PC-Glut-1; FabGennix). Lines E and F visualize GLUT-1 expression of TSH-untreated FRTL-5 cells (E, 3.7 μg; F, 7.4 μg of protein, respectively). The ratio of GLUT-1 signal intensity between TSH-treated (+TSH) and -untreated (−TSH) cells in membrane preparations of equal whole protein mass was determined to be 1.60 ± 0.07 ($n = 3$).

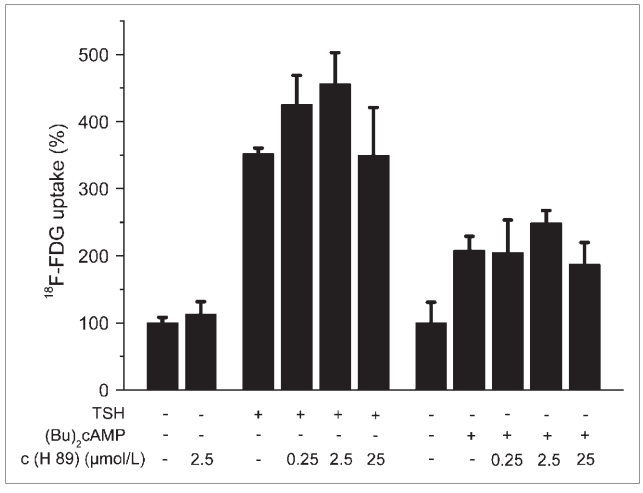

FIGURE 5. Effect of selective PKA inhibitor H89 on $(Bu)_2$cAMP- and TSH-induced ^{18}F-FDG uptake in FRTL-5 cells. H89 did not produce a significant effect on ^{18}F-FDG uptake at concentrations between 0.25 and 25 μmol/L ($P > 0.05$, $n = 4$). Values are mean ± SD of 1 typical experiment performed in quadruplicate. Three independent similar experiments yielded qualitatively identical results. c = concentration.

DISCUSSION

Glucose metabolism in radioiodine-negative metastases of DTC may still be increased by TSH, as demonstrated by ^{18}F-FDG PET in vivo. One possible explanation for this phenomenon may be the differential regulation of signal transduction pathways downstream to the TSH receptor. Therefore, we studied the effect of TSH, of $(Bu)_2$cAMP, and of inhibitors of PI3-kinase and of PKA on ^{18}F-FDG and radioiodide uptake in the same thyroid cellular model.

Recently, we reported the dependence of ^{18}F-FDG uptake on TSH concentration in primary cultures of human thyroid cells (*10*). Thyroid specimens from humans are difficult to obtain. Furthermore, the cell yields reached by their cultivation are low and the behavior of the thyrocytes then proliferating in culture may vary considerably. To circumvent these drawbacks, a more stable in vitro model was needed for our study on signal transduction of radiopharmaceutical uptake in the thyroid. The in vitro models most frequently used to study thyroid biology are immortalized rat thyroid cell lines, such as FRTL-5 (*17*), WRT (*20*), and PC Cl3 (*21*). Among these, FRTL-5 cells exhibit properties ascribed to normal differentiated thyrocytes, such as TSH dependence of growth (*21*) and of differentiated functions including GLUT-1 regulation (*19,22,23*), iodide uptake via the Na^+/I^- symporter (NIS) (*24,25*), and transcription of the genes for thyroglobulin and thyroperoxidase (*26*). FRTL-5 cells thus constitute, by far, the most frequently used thyroid cell line for in vitro studies of thyroid biology (*27*) and also of the TSH dependency of glucose and iodide metabolism (*19,22–25*). Nevertheless, a study comparing the signal transduction of the uptake of the PET radiopharmaceutical ^{18}F-FDG with that of radioiodide in the same subclone of FRTL-5 cells has, to our knowledge, not been reported previously. Studying both parameters in the same subclone of this cell line is important, as there is some variation of biologic behavior between different FRTL-5 batches (*28*). Moreover, it is a well-known phenomenon of the FRTL-5 cell line to spontaneously generate variants with altered TSH dependence when repeatedly passaged, as explicitly discussed in the review of Kimura et al. (*27*).

We observed that the uptake of ^{18}F-FDG and radioiodide by FRTL-5 cells are stimulated by TSH and the cAMP analog $(Bu)_2$cAMP. This confirms data in the literature and the classical view that TSH binding to its receptor activates adenylate cyclase via $G_{\alpha s}$ proteins.

TSH stimulated ^{18}F-FDG uptake by a factor of 2.6, whereas it increased GLUT-1 expression, as determined by Western blotting, only by a factor of 1.6. However, the overall uptake of ^{18}F-FDG in FRTL-5 cells after 24 h of treatment with TSH may not only be due to increased GLUT-1 protein synthesis but also due to changes in hexokinase activity, such as shown for increased FDG uptake by nitric oxide in endothelial cells (*5*). In addition, GLUT-1 translocation from an intracellular pool to the plasma membrane, as demonstrated by Filetti et al. (*29*) and Samih et al. (*19*), could explain the observed difference of GLUT-1 content and increase in ^{18}F-FDG uptake.

Increases in the intracellular concentration of cAMP stimulate PKA; therefore, PKA inhibition by H89 should inhibit the effects of TSH, as it was demonstrated by the abolition of the TSH-induced increase in radioiodide uptake by FRTL-5 cells, in agreement with reports in the literature (*25*). However, in the same subclone of FRTL-5 cells, H89 had no effect on the TSH- and $(Bu)_2$cAMP-mediated increase of ^{18}F-FDG uptake.

Besides TSH, insulin-like growth factor 1 (IGF-1) is the most important regulator of thyroid function; its action is mediated predominantly by PI3-kinase. Interestingly, wortmannin, an irreversible inhibitor of PI3-kinase, blocked the effect of TSH and $(Bu)_2$cAMP on ^{18}F-FDG uptake in FRTL-5 cells and had only little effect on the accumulation of radioiodide.

These results are noteworthy for 2 reasons: First, they prove the existence of a cross-talk mechanism between PI3-kinase responding primarily to the IGF-1 receptor, on the one hand, and cAMP and TSH classically transducing to PKA-mediated pathways, on the other hand. Second, they suggest that a possible upregulation of the PI3-kinase pathway could in principle occur and serve as an explanation for the discrepancy between uptake of radioiodide and ^{18}F-FDG detected by PET in DTC metastases.

The notion that the signal transduction pathways originating at the IGF-1 receptor and at the TSH receptor do not function independently from each other is increasingly entertained in the literature: For example, the TSH-induced cell proliferation within the thyroid gland has been described to depend on the presence of additional growth factors such as IGF-1 (*27*). Further evidence for the role of PI3-kinase in mediating the effect of cAMP in FRTL-5 cells was provided by studies using wortmannin for inhibition of cAMP-induced phosphotyrosine production (*30,31*). Another report revealed increased binding of tyrosine-phosphorylated insulin receptor substrate-1 to the p85 subunit of PI3-kinase by cAMP pretreatment (*32*). Furthermore, involvement of PI3-kinase in GLUT-1 translocation after pretreatment with TSH or insulin has also been demonstrated (*19,22,33,34*). However, a direct stimulation of PI3-kinase by cAMP or PKA is as yet unproven (*35,36*).

The Ras proteins have a central role in the control of cell growth and differentiation (*37*). Studies in transfected rat thyroid cells have shown that cAMP may activate ectopically expressed Ras (*37*), so that Ras may be considered a PKA-independent effector of TSH receptor stimulation. PI3-kinase activation has been described to be necessary for Ras-induced proliferation in human thyrocytes (*38*). Therefore, activation of PI3-kinase by Ras may be the pathway responsible for the TSH- and cAMP-induced increase in ^{18}F-FDG uptake observed in our subclone of FRTL-5 cells, although direct proof of this effect is still missing in the literature.

Clearly, the differential regulation of uptake of ^{18}F-FDG and radioiodide demonstrated herein in a cellular thyroid model could serve as a possible explanation for the maintenance of the TSH dependency of ^{18}F-FDG uptake in radioiodine-negative DTC metastases demonstrated by PET in vivo. This hypothesis receives some support by the observation that the most obvious explanation for this phenomenon—namely, that of a loss of the NIS in the course of neoplastic dedifferentiation—may not be true, as NIS overexpression has been demonstrated immunohistochemically in a significant number of thyroid carcinomas (*39*). An involvement of Ras as a potential relay station in the transduction pathway governing ^{18}F-FDG uptake, but not that of radioiodide, is also interesting in view of the observation that Ras mutations are frequently detected in thyroid carcinomas.

Nevertheless, alternative explanations for the so-called flip–flop phenomenon also seem possible: So could the NIS expressed in thyroid cancer cells be afunctional, for example, by a defect in NIS targeting to the plasma membrane, as suggested by Dohán et al. (*40*). Furthermore, in this context, it is also important to acknowledge that the extrapolation of findings in cell cultures to the in vivo situation is always problematic. As a well-differentiated cell line, FRTL-5 cells may not be representative of thyroid carcinomas that are also a heterogeneous group of neoplasms. Moreover, FRTL-5 cells are a rat cell line and differences between species with regard to the regulation of thyroid cell proliferation have been demonstrated repeatedly.

CONCLUSION

Our findings confirm the existence of a cross-talk mechanism between PI3-kinase responding primarily to the IGF-1 receptor, on the one hand, and cAMP and TSH classically transducing to PKA-mediated pathways, on the other hand. On the basis of our in vitro experiments we conclude that TSH-induced ^{18}F-FDG uptake in FRTL-5 rat thyroid cells is mediated significantly through the PI3-kinase–dependent pathway, whereas radioiodide uptake is mediated primarily by PKA. It is clear that, on the basis of experiments performed using a cell line, direct conclusions concerning the function of the thyroid cells in vivo cannot be made. Nevertheless, this experimental cell model served as a model for the elucidation of intracellular signal transduction pathways in thyroid cells. It is tempting to speculate whether the observed difference in the uptake mechanisms of ^{18}F-FDG and radioiodide could serve as an additional explanation for the so-called flip–flop behavior of radiopharmaceutical uptake values observed in vivo.

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