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In vitro characterization of the thyroidal uptake of *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine

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Abstract

Objectives: Positron emission tomography (PET) using *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET) has been successfully employed in the diagnostic workup of brain tumors. Knowledge on the mechanisms of the uptake of radiolabeled amino acids into thyroidal tissues and well-differentiated thyroid carcinomas is limited. We therefore studied several factors potentially governing the uptake of FET in the rat thyroid cell line FRTL-5 in comparison with thyroid tumor cell lines of human origin.

Methods: FET uptake was determined in thyroid-stimulating hormone (TSH)-stimulated and TSH-deprived FRTL-5 cells, as well as in the cell lines U-138 MG (human glioblastoma), Onco DG-1 (human papillary thyroid carcinoma) and ML-1 (human follicular thyroid carcinoma). The TSH responsiveness of cells was measured by the incubation of TSH-treated and untreated control cells with 2-[^{18}F]fluoro-2-deoxyglucose (FDG). All cellular tracer uptake values were related to total protein mass and expressed as percentage per milligram. For countertransport studies, FRTL-5 cells were exposed to 10–300 μM tyrosine methyl ester. TSH-stimulated and TSH-deprived FRTL-5 cells were incubated with 100 kBq/ml FET for 20 min. 2-Aminobicyclo-[2,2,1]heptane-2-carboxylic acid (BCH), α-(methylamino)-isobutyric acid, L-serine and tryptophan were used as competitive inhibitors of FET uptake. All inhibition experiments were repeated with the human thyroid carcinoma cell lines to obtain comparative FET uptake values.

Results: The FET uptake was 155±30%/mg in FRTL-5 cells (n=6), 108±14%/mg in U-138 MG cells (n=6), 194±60%/mg in ML-1 cells (n=9) and 64±23%/mg in Onco DG-1 cells (n=6) under identical incubation conditions. Preloading with tyrosine methyl ester increased cellular FET uptake dose dependently in FRTL-5 cells (165±25%, n=6). While TSH increased the uptake of FDG in FRTL-5 cells by sixfold, there was no TSH effect on FET accumulation. FET uptake by TSH-treated FRTL-5 cells was sodium independent and significantly inhibited by BCH (91.4±3.0%, n=9), tryptophan (94.8±1.6%, n=8) and serine (83.2±10.8%, n=12). TSH-starved FRTL-5 cells had a sodium-dependent component with a similar inhibition pattern. Onco DG-1 mainly confirmed the inhibition pattern of FET uptake in FRTL-5 cells, reflecting System-L-mediated FET uptake that was blocked by BCH and serine (72–85%, n=9). ML-1 cells revealed a pronounced sodium-dependent FET uptake that was inhibited by tryptophan (70±10%, n=9, P<.05) in the presence and in the absence of sodium, suggesting a contribution of alternative amino acid carriers.

Conclusion: FET uptake by FRTL-5 cells is not TSH dependent. FET uptake by FRTL-5 cells seems to be mainly mediated by a carrier exhibiting the characteristics of the System L amino acid transporter. FET uptake in thyroid cells and thyroid carcinoma cells was in the same range as that in a glioblastoma cell line. This encourages further research efforts towards the clinical evaluation of FET for the diagnostic workup of well-differentiated thyroid carcinomas.

Keywords: *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine; FET; amino acid transport; Thyroid; FRTL-5

1. Introduction

Positron emission tomography (PET) using 2-[^{18}F]fluoro-2-deoxyglucose (FDG) can be used to localize metastases of well-differentiated thyroid carcinomas, which have lost their ability to concentrate radioactive iodine [1]. FDG is, however, not exclusively accumulated by neoplastic cells, but also by activated macrophages and endothelia [2–4]. Therefore, FDG-PET cannot reliably differentiate neoplastic lesions from inflammatory lesions, which decreases the diagnostic specificity of this technology.

Evidence from in vitro studies and animal models suggests that radiolabeled amino acids are more specific for neoplastic tissues than is FDG [5–8]. Progress in the clinical use of these compounds has been slowed by the

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absence of ^{18}F-labeled amino acids that can be produced by an automated radiosynthesis with high radiochemical yields [9]. However, in 1999, *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET) was introduced as a tracer of amino acid transport that does not suffer from these limitations [10,11].

FET-PET has been successfully employed in the diagnostic workup of brain tumors [12–14]. Moreover, the first study to investigate the use of FET-PET in peripheral tumors reported better distinction between squamous cell carcinomas and inflammatory tissues with FET-PET than with FDG-PET [15].

In vitro studies elucidating the transport characteristics of FET are as yet scarce [16]. So far, it has been demonstrated that FET transport into the brain is stereoselective [10]. Moreover, cellular FET uptake is usually attributed to transport via System L, since FET accumulation was inhibited by 2-aminobicyclo-[2,2,1]heptane-2-carboxylic acid (BCH) in SW 707 colon carcinoma cells [17]. BCH is generally considered to be a selective inhibitor of the Na^+-independent amino transport System L, but also inhibits the Na^+-dependent System $B^{0,+}$ [18]. Accordingly, Langen et al. [19] described the sodium-dependent transport of FET in the rat glioma cell line F98 that could be ascribed mainly to System $B^{0,+}$, besides sodium-independent transport via System L. More recently, Stöber et al. [20] provided evidence that System-L-mediated FET uptake allows the differentiation of tumor and inflammation in vitro.

The aims of this study were to investigate the mechanisms governing FET uptake by the rat thyroid cell line FRTL-5 and to compare such cell line with thyroid carcinoma cell lines of human origin. In particular, we aimed at analyzing the potential role of thyroid-stimulating hormone (TSH) in this variable since TSH has been shown to have a stimulatory effect on the thyroidal uptake of several other radiopharmaceuticals commonly used in the diagnostic workup of well-differentiated thyroid cancer [21–23]. For further characterization of amino acid transport systems involved in thyroidal FET uptake, we used a well-established battery of inhibitory amino acids in TSH-stimulated and TSH-deprived FRTL-5 cells.

2. Materials and methods

2.1. Reagents

FDG was purchased from PET Net GmbH (Erlangen, Germany). BCH, α-(methylamino)-isobutyric acid (MeAIB), tryptophan, L-serine (Ser), choline chloride and bovine TSH were supplied by Sigma-Aldrich (Deisenhofen, Germany). Dulbecco's modified Eagle's medium (DMEM), fetal calf serum (FCS), phosphate-buffered saline (PBS) and trypsin/EDTA were obtained from Invitrogen/Gibco (Germany).

2.2. Radiosynthesis of FET

FET was synthesized by an automated synthesis module consisting of a Simatic S7-200 device and a ProTool/Pro software (Siemens, Germany), following the procedure described by Wester et al. [10]. FET was obtained as an intravenous injectable PBS (pH 5.5) containing 15–64 μg/ml tyrosine.

2.3. Cell culture

As reported previously [23], the rat thyroid cell line FRTL-5 was obtained from the American Type Culture Collection (ATCC; Bethesda, MD) and grown in a 6H medium based on Coon's F12 solution (Sigma, Deisenhofen, Germany) supplemented with 5% FCS and a six-hormone mixture containing 10 mg/L insulin, 5 mg/L transferrin, 10 μg/L somatostatin, 10 nM hydrocortisone, 10 μg/L glycyl-L-histidyl-L-lysine acetate and 1 U/L bovine TSH. Cells were routinely passaged every 3–4 days. Approximately 7×10^4 cells/ml in 6H medium were replated in 12-multiwell culture plates 4–5 days before initiating FET uptake studies. TSH deprivation was performed by replating 1.4×10^5 cells/ml in 6H medium and by changing to 5H medium (without TSH) after 24 h. TSH deprivation was continued for 3–4 days. The viability of FRTL-5 cells was verified by trypan blue staining, and TSH receptor expression was proven by immunocytochemical staining using TSH receptor antibody-1 (TSHR mouse monoclonal antibody, clone 4C1/E1/G8; Lab Vision Co., Westinghouse) using the universal DAKO-APAAP Kit (DAKO, Hamburg, Germany), as described in detail previously [23].

The human glioblastoma cell line U-138 MG (ATCC), which was used as a positive control in FET uptake experiments, was grown in DMEM supplemented with 10% FCS until it reached subconfluence. The human papillary thyroid carcinoma cell line Onco DG-1 was purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ) (ACC507; Braunschweig, Germany) and grown in RPMI 1640 medium supplemented with 10% FCS. The human follicular thyroid carcinoma cell line ML-1 was obtained from the DSMZ (ACC464) and cultured in DMEM containing 2 mM L-glutamine, 100 μM sodium pyruvate and 10% FCS.

2.4. Measurement of FDG uptake

FDG uptake experiments were performed as previously described [23], with slight modifications. In brief, cells were seeded at a density of 40,000 cells/cm^2 in a cell culture medium, as stated above. Prior to FDG uptake experiments, TSH deprivation of FRTL-5 cells was performed by keeping the cells in a deprivation medium (6H medium without TSH) for 3 days. ML-1 and Onco DG-1 cells were serum starved in their respective culture medium containing 1% FCS for 2 days. Subsequently, TSH-treated carcinoma cells were obtained by treatment with 1 mU/ml TSH for 24 h. FDG (0.5–1 MBq, 10 μl) was added to each 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. After washing the cell layers with cold PBS, 0.5 ml of 0.1 M