SYNTHESIS AND FIRST EVALUATION OF NEW ^{18}F-LABELLED SULFONYLUREAS FOR THE DETERMINATION OF THE BETA-CELL STATUS IN VIVO

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Summary:
The syntheses and first in vitro evaluations for two fluoride bearing sulfonylurea derivatives are reported. Firstly, the tolbutamide derivative 1-[4-(2-[^{18}F]fluoroethoxy)benzenesulfonyl]-3-butyl urea (2-[^{18}F]fluoroethyl-tolbutamide) could be labeled efficiently with [^{18}F]fluoride. Subsequently, the glibenclamid derivative N-(2-(4-(N-((cyclohexylamino)carbonyl)sulfonyl-amino)phenyl)ethyl) 2-(5-chloro-2-[^{18}F]fluorethoxy)phenyl) formamide (2-[^{18}F]fluoroethyl-glibenclamide) was labeled with [^{18}F]fluoride in high yields. Its ability to induce insuline secretion from rat beta-cells in relation to the original glibenclamide was determined.

Introduction:
Diabetes mellitus comprises a heterogeneous group of disorders characterized by high blood glucose levels. Two major types of diabetes mellitus have been defined: type 1 (insulin-dependent diabetes mellitus, IDDM), and type 2 (non-insulin-dependent diabetes mellitus, NIDDM). Although hyperglycemia is the common denominator of both IDDM and NIDDM, the etiology and pathophysiology of these syndromes are distinct. IDDM is a chronic autoimmune disease characterized by the selective destruction of insulin-producing β-cells of the islets of Langerhans. When autoimmune destruction affects more than 90% of the β-cell mass, the resulting insulin deficiency culminates into development of overt hyperglycemia. With NIDDM on the other hand, the pancreatic β-cells are initially intact, and the disease is associated with insulin resistance and loss of β-cell function, and eventual insulin-dependency [1].

The aim of this study was to synthesize β-cell-specific positron emitting radiolabeled sulfonylurea derivatives such as 2-[^{18}F]fluoroethyl-tolbutamide (1) and 2-[^{18}F]fluoroethyl-glibenclamide to image the β-cell mass *in vivo* via positron emission tomography (PET). Tolbutamide with a K_i of 25-55 μM and glibenclamide with a K_i of 0.7-7 nM are sulfonylurea agents used to stimulate insulin secretion in type 2 diabetic patients [2]. We intend to determine the efficacy of these radiolabeled agents in visualizing and quantifying β-cell concentrations in the pancreas of normal non-human primates by PET.

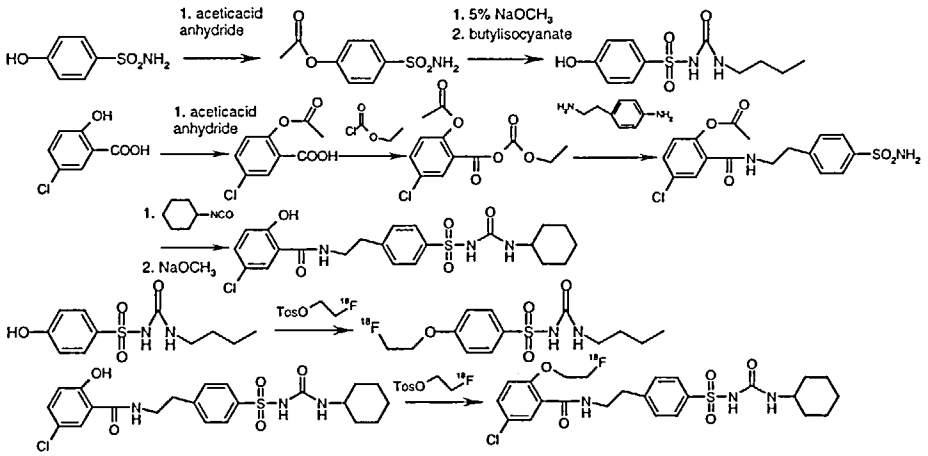

Fig. 1: Synthesis of the labeling precursors and their radioactive labeling

Results and discussion:

First the ^{19}F-compounds of the described sulfonylurea derivatives were synthesized for testing their ability to stimulate insulin secretion from rat beta-cells. The respective labeling precursors for the radiolabeling of the tolbutamide and glibenclamide derivatives were synthesized as shown in figure 1. All compounds were verified with common analytical methods such as ^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR, mass spectroscopy and elemental analysis.

Radioactive labeling could easily be achieved by HO-^{18}F-fluoroalkylation of the labeling precursors with 2-[^{18}F]fluoroethyltosylate in DMSO at 80°C in radiochemical yields ranging from 80-90% (fig. 1). The overall radiochemical yields were between 60-70% (uncorrected).

In vitro evaluation:
For testing the *in vitro* function of the glibenclamide derivative 2-[^{19}F] fluoroethyl-glibenclamide a standardized batch stimulation was performed. Adult rat islets were isolated by collagenase digestion and purified by a density gradient. For each sample ten islets were used (equal in size and shape) for a culture-insert with a membrane of 3 µm pore size. First the basal insulin secretion was tested by culturing the islets with normo-glycemic culture-media (RPMI 1640 + D-glucose 100mg/dl + 10% FCS + P/S) for 1 hour at 37°C. After the culture period the media were collected and stored at –20°C. The inserts with islets were transferred to normo-glycemic culture-media with several concentrations of the glibenclamide derivative and cultured for a second, stimulated period. As a positive control several inserts with islets were cultured with a hyperglycemic culture-media (RPMI 1640 D-glucose 300mg/dl + 10% FCS + P/S) only. For the negative control normo-glycemic culture-media (RPMI 1640 D-glucose 100mg/dl + 10% FCS + P/S) with a diluted-solution but without the glibenclamide derivative was used. The insulin content of each probe was quantified by a rat-insulin-ELISA. The stimulation effect (in %) was calculated as stimulated insulin secretion divided by basal insulin secretion * 100 (tab. 1)
We could detect nearly the same stimulation effect of 0.25ng/ml 2-[^{19}F]fluoro-ethyl-glibenclamide as in the hyperglycemic positive control. The evaluation of 2-[^{19}F]fluoroethyl-tolbutamide is under investigation.

Tab 1: Effects on insulin secretion of 2-[^{19}F]fluoroethyl-glibenclamide

	Concentration of 2-[^{19}F]fluoroethyl-glibenclamide [ng/ml]				
	0.025	0.25	2.5	Pos. control	Neg. control
Stimulation effect [%]	291.6	471.1	160.0	533.4	115.2
SD [+/- %]	62.2	101.5	9.8	80.6	13.1

Conclusion:
The syntheses of two ^{18}F-labeled sulfonylurea derivatives, 2-[^{18}F]fluoroethyl-tolbutamide and 2-[^{18}F]fluoroethyl-glibenclamide in overall radiochemical yields between 60-70% were reported for their use as beta-cells imaging agents. The non radioactive ^{19}F-standard compound 2-[^{19}F]fluoroethyl-glibenclamide was successfully evaluated in comparison to glibenclamide for the effect on insulin secretion. The required labeling precursors were obtained via multi-step synthetic routes and validated with common analytical methods.

References :
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