ELSEVIER

Nuclear Medicine and Biology 31 (2004) 483–491

NUCLEAR MEDICINE — AND — BIOLOGY

www.elsevier.com/locate/nucmedbio

Synthesis and evaluation of fluorine-18 labeled glyburide analogs as β-cell imaging agents

A. Schmitz[a], C.-Y. Shiue[a,*], Q. Feng[a], G.G. Shiue[a], S. Deng[e], M.T. Pourdehnad[a], R. Schirrmacher[b], M. Vatamaniuk[c], N. Doliba[c], F. Matschinsky[c], B. Wolf[d], F. Rösch[b], A. Naji[e], A.A. Alavi[a]

[a]*Department of Radiology, University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104, USA*
[b]*Institut für Kernchemie, Johannes Gutenberg Universität Mainz, Fritz Strassmann-Weg 2, 55128 Mainz, Germany*
[c]*Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104, USA*
[d]*Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA*
[e]*Department of Surgery, University of Pennsylvania, Philadelphia, PA 19104, USA*

Received 25 October 2003; received in revised form 28 November 2003; accepted 3 December 2003

Abstract

Glyburide is a prescribed hypoglycemic drug for the treatment of type 2 diabetic patients. We have synthesized two of its analogs, namely *N*-{4-[β-(2-(2′-fluoroethoxy)-5-chlorobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (2-fluoroethoxyglyburide, 8b) and *N*-{4-[β-(2-(2′-fluoroethoxy)-5-iodobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (2-fluoroethoxy-5-deschloro-5-iodoglyburide, 8a), and their fluorine-18 labeled analogs as β-cell imaging agents. Both F-18 labeled compound 8a and compound 8b were synthesized by alkylation of the corresponding multistep synthesized hydroxy precursor 4a and 4b with 2-[^{18}F]fluoroethyl tosylate in DMSO at 120°C for 20 minutes followed by HPLC purification in an overall radiochemical yield of 5-10% with a synthesis time of 100 minutes from EOB. The octanol/water partition coefficients of compounds 8a and 8b were 141.21 ± 27.77 (n = 8) and 124.33 ± 21.61 (n = 8), respectively. Insulin secretion experiments of compounds 8a and 8b on rat islets showed that both compounds have a similar stimulating effect on insulin secretion as that of glyburide. *In vitro* binding studies showed that ~2% of compounds 8a and 8b bound to βTC3 and Min6 cells and that the binding was saturable. Preliminary biodistribution studies in mice showed that the uptake of both compounds 8a and 8b in liver and small intestine were high, whereas the uptake in other organs studied including pancreas were low. Additionally, the uptake of compound 8b *in vivo* was nonsaturable. These results tend to suggest that compounds 8a and 8b may not be the ideal β-cell imaging agents.

Keywords: 2-[^{18}F]fluoroethoxyglyburide; 2-[^{18}F]fluoroethoxy-5-iodoglyburide; sulfonylurea receptors; β-cell imaging agents; diabetes

1. Introduction

Diabetes mellitus is a major public health problem, affecting more than 5% of the world's population and 25% of persons over 60 years of age. 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 and type 2. Although hyperglycemia is the common denominator of both type 1 and type 2 diabetes, the etiology and syndromes are distinctly different. Type 1 accounts for about 10% of all diabetes; it is a chronic autoimmune disease characterized by the selective destruction of insulin-producing β-cells of the pancreas, leading to a near total deficiency in insulin secretion. When autoimmune destruction affects more than 90% of the β-cells mass, the resulting insulin deficiency culminates into the development of overt hyperglycemia. In contrast, type 2 is the most common form of diabetes, accounting for more than 90% of cases; it is a chronic, progressive metabolic disorder of carbohydrate and lipid metabolism. Type 2 diabetes is caused by two physiological defects: resistance to the action of insulin, combined with a deficiency in insulin secretion [1,2]. There are two types of drug therapy for type 2 diabetes mellitus: 1) oral agents (sulfonylureas, biguanides, thiazolidinediones, α-glucosidase inhibitors,

* Corresponding author. Tel.: (215) 662-7797; fax: (215) 662-7551.
E-mail address: Shiue@rad.upenn.edu (C.-Y. Shiue).

0969-8051/04/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.nucmedbio.2003.12.003

meglitinide analogs); and 2) parenteral agents (insulin, insulin analogs, amylin agonists, glucagon-like peptide, glucagon antagonists) [3]. Fewer than half of all diabetics receive treatment, and of these only a very small proportion achieve a level of glucose control that is sufficient to avoid the morbidity associated with the disease. To date, there have been no reported techniques to image the endocrine pancreas. The basis of our inability to image the endocrine pancreas has been due to the unavailability of a marker specific for islet β-cells. In the context of type 1 diabetes mellitus, the chronic and progressive loss of β-cells resulting from autoimmune destruction has led to concerted efforts to prevent further loss of β-cells by autoantigen-specific immunotherapy of prediabetic patients [4]. In addition, a number of novel strategies for therapy of diabetes mellitus are based on replication of β-cells and islet transplantation. Since we have an ongoing islet transplantation program [5,6], it is of considerable importance to have a reliable noninvasive method to monitor the progressive loss of β-cells mass during the silent phase of prediabetes or transplanted islet mass.

Positron emission tomography (PET) coupled with appropriate radiotracers has the unique capability of noninvasively measuring biochemical and metabolic processes. Sulfonylureas are antidiabetic agents that block pancreatic ATP-sensitive potassium channels, located at the insulin-producing β cells of the islets of Langerhans, either directly or via a plasma membrane-associated protein, resulting in an increase of intracellular calcium ion and consequent insulin secretion [7,8]. Therefore, if sulfonylureas were labeled with a positron emitter, they may serve as β-cell imaging agents. Tolbutamide and glyburide are hypoglycemic drugs that bind to sulfonylurea receptors (SUR) [9-11] in HIT-β cells with a wide ranges of affinities (K_i= 25-55 μmol/L [12] and 0.7-7 nmol/L [13] for tolbutamide and glyburide, respectively). The uptake of [^{3}H]glyburide has been shown to be proportional to the number of β-cells and is saturable [14]. The B_{max} of SUR in the pancreatic β-cells and mouse islets are 1,400-1,600 fmol/mg protein [15]. In addition, the fluoro analog of tolbutamide, 1-[(*p*-fluorobenzenesulfonyl)]-3-butylurea, was reported to have a similar hypoglycemic potency as tolbutamide [16]. Therefore, we have synthesized 1-(4-(2-[^{18}F]fluoroethoxy)-benzenesulfonyl)-3-butylurea [17], 1-[(*p*-[^{18}F]fluorobenzenesulfonyl)]-3-butylurea (*p*-desmethyl-*p*[^{18}F]fluorotolbutamide) and *N*-{4-[β-(2-(2′- [^{18}F] fluoroethoxy-5-chlorobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea ([^{18}F]fluoroethoxyglyburide, 8b) as potential β-cell imaging agents [18]. We report herein the synthesis of another glyburide analog, *N*-{4-[β-(2-(2′-fluoroethoxy)-5-iodobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (2-fluoroethoxy-5-deschloro-5-iodoglyburide, 8a) and the evaluation of these two glyburide analogs (8a and 8b) (Fig. 1) as potential β-cell imaging agents. Part of this study has previously appeared in abstract form [19].

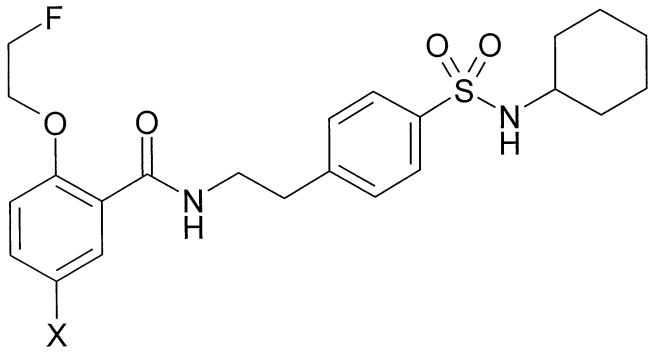


Fig. 1. Chemical structures of 2-fluoroethoxy-5-iodoglyburide (**8a**) and 2-fluoroethoxyglyburide (**8b**).

2. Methods and materials

Ethylene glycol di-*p*-tosylate, 5-chlorosalicyclic acid, 5-iodosalicyclic acid, 1-bromo-2-fluoroethane, ethyl chloroformate, 4-(2-aminoethyl)benzenesulfonamide, cyclohexyl isocyanate, copper(I) chloride and borontrifluoride etherate were purchased from Aldrich Chemical Company (Milwaukee, WI) and used without further purification. C_{18} Sep-Pak cartridges were obtained from Waters Chromatography Division, Millipore Corporation (Milford, MA). Radioactivity was determined using a calibrated ion chamber (Capintec CRC-745, Capintec, Inc., Ramsey, NJ) and a sodium iodide well counter (Packard, Gamma Counter 5000 Series, Packard Instrument Company, Meriden, CT).

High-performance liquid chromatography (HPLC) analyses were carried out with a Sonntek liquid chromatograph equipped with both UV and radioactivity monitors and C_{18} columns (Phenomenex, Luna 2, 10 $\times$ 250 mm). The elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA). Elemental compositions were within $\pm$0.3% of the calculated values. Melting points were determined on a MEL-Temp II apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker DPX 200 spectrometer. Chemical shifts (δ) are expressed in parts per million relative to internal tetramethylsilane.

All animal studies were carried out according to protocol approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

2.1. Synthesis of precursors and authentic samples

The precursor *N*-{4-[β-(2-hydroxy-5-chlorobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (4b) and the authentic sample *N*-{4-[β-(2-(2′-fluoroethoxy)-5-chlorobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (8b) were synthesized as described previously [18]. The other precursor, *N*-{4-[β-(2-hydroxy-5-iodobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (4a) [20] and the other authentic sample,

N-{4-[β-(2-(2′-fluoroethoxy)-5-iodobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (8a) were prepared by a multistep synthesis as follows.

*2.1.1. Synthesis of precursor N-{4-[β-(2-hydroxy-5-iodobenzenecarboxamido)ethyl]-benzenesulfonyl}-N′-cyclohexylurea (***4a***):*

*2.1.2. 2-Acetoxy-5-iodobenzoic acid (***2a***)*

To a solution of 5-iodosalicylic acid (**1a**) (13.2 g, 50 mmol) and acetic anhydride (15.3 g, 150 mmol), a few drops of concentrated sulfuric acid was added. The solution was refluxed for 30 minutes, cooled to room temperature, and poured into 100 mL of ice water. The resulting yellow solid was filtered and recrystallized from ethanol to give 30.5 g (45.5%) of compound **2a** as a white solid, mp 161-162°C. ^{1}H-NMR ($CDCl_3$): δ 8.4 (d, 1H); 7.9 (dd, 1H); 6.9 (d, 1H); 2.3 (s, 3H).

*2.1.3. 4-[β-(2-Acetoxy-5-iodobenzenecarboxamido)ethyl] benzenesulfonamide (***3a***)*

To a solution of compound 2a (20.1 g, 65 mmol) in 150 mL of DMF, triethylamine (9.1 mL 65 mmol) was added. The solution was cooled to 0°C and then ethyl chloroformate (6.3 mL, 65 mmol) was added. The resulting white suspension was stirred at 0°C for 30 minutes and then a solution of *p*-(β-aminoethyl)benzenesulfonamide (13.0 g, 65 mmol) and triethylamine (9.1 mL, 65 mmol) in 100 mL of DMF was added dropwise to the cooled solution. The white suspension was continued to stir at room temperature for an additional 4 hours and evaporated to dryness. The resulting yellowish oil was mixed with 0.2 N HCl (200 mL). The resulting beige precipitates were filtered, dried, and recrystallized from ethanol to give 4-[β-(2-acetoxy-5-iodobenzenecarboxamido)ethyl]benzenesulfonamide (3a) (22.1 g, 69.7%), mp 190-191°C. ^{1}H-NMR ($CDCl_3$): δ 8.5 (t, 1H); 8.1 (d, 1H); 7.8 (d, 2H); 7.7 (dd, 1H); 7.4 (d, 2H); 6.7 (d, 1H); 6.3 (d, 1H); 3.5 (m, 2H); 2.9 (m, 2H); 2.3 (s, 3H). Anal. ($C_{17}H_{17}IN_2O_5S$) C, H, N.

*2.1.4. N-{4-[β-(2-Hydroxy-5-iodobenzenecarboxamido) ethyl]benzenesulfonyl}-N′-cyclohexylurea (***4a***)*

To a solution of compound 3a (4.8 g, 10 mmol) in 30 mL of DMF, copper (I) chloride (99 mg, 1 mmol) was added. The resulting greenish solution was stirred at room temperature while cyclohexyl isocyanate (1.5 g, 12 mmol) was added dropwise over the course of 30 minutes. The greenish solution was continued to stir in the dark at ambient temperature for 2 days and then was poured into 150 mL of ice water. The greenish precipitates were filtered by suction, dried, and recrystallized twice from ethyl acetate to give *N*-{4-[β-(2-acetoxy-5-iodobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea as white crystals (3.6 g, 58%), mp 178-179°C. ^{1}H-NMR ($CDCl_3$): δ 12.5 (s, 1H); 10.4 (s, 1H); 8.9 (s, 1H); 8.5 (t, 1H); 8.1 (d, 1H); 7.8 (d, 2H); 7.7 (dd, 1H); 7.4 (d, 2H); 6.7 (d, 1H); 6.3 (d, 1H); 3.5 (m, 2H); 2.9 (m, 2H); 2.3 (s, 3H); 1.6 (m, 5H); 1.1 (m, 6H).

A freshly prepared sodium methoxide solution in methanol was added to a solution of *N*-{4-[β-(2-acetoxy-5-iodobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (3.6 g, 5.9 mmol) in 100 mL of methanol. The solution was stirred at ambient temperature for 4 hours, poured into ice water, and acidified with 1N HCl. The white precipitates were filtered by suction, dried, and recrystallized from ethanol to give the title compound 4a as white powders (2.9 g, 89.5%), mp 208-209°C. ^{1}H-NMR ($CDCl_3$): δ 12.5 (s, 1H); 10.4 (s, 1H); 8.9 (s, 1H); 8.5 (t, 1H); 8.1 (d, 1H); 7.8 (d, 2H); 7.7 (dd, 1H); 7.4 (d, 2H); 6.7 (d, 1H); 6.3 (d, 1H); 3.5 (m, 2H); 2.9 (m, 2H); 1.6 (m, 5H); 1.1 (m, 6H); Anal. ($C_{22}H_{26}IN_3O_5S$) C, H, N.

*2.2. Synthesis of authentic sample N-{4-[β-(2-(2′-fluoroethoxy)-5-iodobenzenecarboxamido)ethyl]benzenesulfonyl}-N′-cyclohexylurea (***8a***)*

*2.2.1. 2-(2′-Fluoroethoxy)-5-iodobenzoic acid (***6a***)*

To a solution of 5-iodosalicylic acid (1a) (2.1 g, 8 mmol) in 60 mL of DMF, sodium hydride (absorbed on mineral oil, 60%) (0.64 g, 16 mmol) was added slowly. The mixture was stirred at room temperature under an inert atmosphere until no more gas evolves. To this clear yellowish solution, 1-bromo-2-fluoroethane (2.2 g, 17 mmol) was added. The solution was stirred at 80°C for 24 hours, cooled to ambient temperature, and evaporated to dryness. The light brownish residue was dissolved in ethyl acetate and extracted with water and brine. The organic phase was dried over $MgSO_4$ and evaporated to dryness. The yellowish oil was purified by column chromatography (Silica gel 60, ethyl acetate/hexanes 19:1) to give 2′-fluoroethyl 2-(2′-fluoroethoxy)-5-iodobenzoate (5a) as a light yellowish solid, mp 71-72°C. ^{1}H-NMR ($CDCl_3$): δ 8.1 (d, 1H); 7.8 (d, 1H); 6.7 (d, 1H); 4.9 (m, 2H); 4.6 (m, 3H); 4.4 (m, 1H); 4.3 (m, 1H); 4.1 (m, 1H). Compound 5a was used for the next step without further purifications.

A solution of compound 5a (2.6 g, 7 mmol) in 50 mL of sodium hydroxide solution (5%) was stirred at ambient temperature for 36 hours and then acidified with 1N HCl. The white precipitates were filtered, washed with water, dried, and recrystallized from ethanol to yield compound 6a as white crystals (1.9 g, 6.1 mmol, 76.6%), mp 129-130°C. ^{1}H-NMR ($CDCl_3$): δ 10.5 (br s, 1H); 8.4 (d, 1H); 7.8 (d, 1H); 6.8 (d, 1H); 4.9 (m, 1H); 4.7 (m, 1H); 4.5 (m, 1H); 4.4 (m, 1H).

*2.2.2. 4-[β-(2-Fluoroethoxy-5-iodobenzenecarboxamido) ethyl]benzenesulfonamide (***7a***)*

To a solution of compound 6a (450 mg, 1.45 mmol) in 50 mL of DMF under an inert atmosphere, triethylamine (0.15 g, 1.5 mmol) was added. The yellowish solution was cooled to 0°C and ethyl chloroformate (0.162 g, 1.5 mmol) was added. The resulting white suspension was continued to stir

at 0°C for 30 minutes and then a solution of *p*-(β-aminoethyl)benzenesulfonamide (300 mg, 1.5 mmol) and triethylamine (0.15 g, 1.5 mmol) in 30 mL of DMF was added dropwise. The mixture was stirred at ambient temperature for 24 hours and evaporated to dryness. The yellowish residue was suspended in 100 mL of 1N HCl. The resulting white precipitates were filtered, dried, and recrystallized from ethanol to give compound 7a as white powders (625 mg, 87.6%), mp 161-163°C. ^{1}H-NMR (DMSO-d_6): δ 8.2 (t, 1H); 7.9 (d, 1H); 7.8 (s, 1H); 7.7 (d; 2H); 7.4 (d; 2H); 7.3 (s; 2H); 6.9 (d, 1H); 4.8 (m, 1H); 4.6 (m, 1H); 4.4 (m, 1H); 4.2 (m, 1H); 3.5 (m, 2H); 2.9 (m, 2H).

*2.3. Synthesis of N-{4-[β-(2-(2′-fluoroethoxy)-5-iodobenzenecarboxamido)ethyl]-benzenesulfonyl}-N′-cyclohexylurea (***8a***)*

Compound 8a was synthesized by the following two methods.

2.3.1. Coupling of compound 7a with cyclohexyl isocyanate

A greenish solution of CuCl (15 mg, 0.15 mmol), compound 7a (625 mg, 1.25 mmol) and cyclohexyl isocyanate (170 mg, 1.35 mmol) in 40 mL of DMF was stirred in the dark, at ambient temperature, and under nitrogen atmosphere for 72 hours. The solution was then poured into 150 mL of ice water. The resulting greenish suspension was stirred vigorously until greenish precipitates were formed. The product was purified by column chromatography (silica gel 60, hexanes/ethyl acetate 19:1, R_f= 0.85) to yield compound 8a as a white powder (405 mg, 52.5%). The product was recrystallized from ethyl acetate, mp 140-142°C. ^{1}H-NMR (DMSO-d_6): δ 0.2 (s, 1H); 8.2 (t, 1H); 7.9 (d, 1H); 7.8 (d, 2H); 7.7 (s; 1H); 7.4 (d; 2H); 6.9 (d, 1H); 6.3 (d, 1H); 4.8 (m, 1H); 4.6 (m, 1H); 4.4 (m, 1H); 4.2 (m, 1H); 3.6 (m, 2H); 2.9 (m, 2H); 1.6 (m, 5H); 1.2 (6H). Anal. ($C_{24}H_{29}FIN_3O_5S \cdot C_4H_8O_2$) C, H, N.

2.3.2. Alkylation of compound 4a with 1-bromo-2-fluoroethane

To a solution of compound 4a (0.260 mmol) in 10 mL of dry CH_3CN and 1 mL of dry DMSO, 270 μL of 1N NaOH was added. After stirring the solution for 10 minutes, 1-bromo-2-fluoroethane (1.250 mmol) was added to the solution. This mixture was kept at 60°C and stirred for several days. The progress of the reaction was monitored with HPLC (Luna 2, C_{18}, 4.6 × 250 mm, CH_3OH 70%, flow 1 mL/min).

After stirring at 60°C for 3 days, the HPLC showed the formation of the desired product 8a. The reaction mixture was concentrated, neutralized with 1N HCl, and injected into semi-preparative HPLC (Phenomenex, Luna 2, C_{18}, 10 × 250 mm, CH_3OH:H_2O, 7:3; 4 mL/min). The fractions that correspond to product 8a and precursor 4a were collected and evaporated to give 10 mg (6.2%) of product 8a and 8 mg (5%) of precursor 4a.

2.4. Synthesis of N-{4-[β-(2-(2′-[^{18}F]fluoroethoxy-5-iodobenzenecarboxamido)ethyl]benzene-sulfonyl}-N′-cyclohexylurea (2-[^{18}F]Fluoroethoxy-5-deschloro-5-iodoglyburide, **8a***)*

Fluorine-18 labeled compound 8a was synthesized in a similar manner as that described for the synthesis of compound 8b [18]. Thus, a solution of precursor 4a (2.5 mg in 10 μL of 1N NaOH and 0.3 mL of DMSO) was added to 1-[^{18}F]fluoro-2-tosylethane in 0.5 mL of CH_3CN, heated at 90°C for 30 minutes and then cooled to room temperature. Water (5 mL) was added and the solution was passed through a C_{18} Sep-Pak. The Sep-Pak was rinsed with two additional 10-mL quantities of water followed by ether (2 × 6 mL). The combined washings were discarded. The crude product 8a was rinsed out with CH_3OH (2 × 3 mL), neutralized with HCl (1N, 10 μL), concentrated to ~1.5 mL and injected into HPLC (Phenomenex, Luna 2, C_{18}, 10 × 250 mm, CH_3OH:H_2O, 7:3; 4 mL/min). The fraction containing compound 8a was collected from 27.5 to 30.5 minutes. The solution was concentrated to 1 mL, 5 mL of saline was added, and the resulting solution was filtered through a 0.22-μm cellulose acetate membrane filter (Millipore) into a multi-injection vial. The radiochemical yield was ~10% and the synthesis time was 100 minutes from EOB. The specific activity was 0.5 Ci/μmol. HPLC analysis (Phenomenex, Luna 2, C_{18}, 4.6 × 250 mm, CH_3OH:H_2O, 7:3; 1 mL/min) showed that the radiochemical purity was >99%.

2.5. Determination of partition coefficient of F-18 labeled compounds 8a and 8b

Octanol/water partition coefficients of F-18 labeled compounds 8a and 8b were measured by adding 5 μL of the compound in saline solution to a 5 mL vial containing 1 mL each of 1-octanol and pH 7.0 phosphate buffer. The vial was capped and vortexed vigorously for 10 minutes at room temperature. After reaching equilibrium, the organic phase was pipetted out and each phase was counted in a NaI well counter. The partition coefficient was calculated as (cpm in 1-octanol)/(cpm in pH 7.0 phosphate buffer).

2.6. Islet isolation and perifusion

Rat islets were isolated using standard collagenase procedure [21] followed by separation of islets from exocrine tissue in Ficoll gradient. Freshly isolated rat islets were placed on nylon filter in a plastic perifusion chamber (Millipore, MA). Perifusion apparatus consisted of computer-controlled HPLC system (Waters 625 LC System) that allowed programmable rates of perfusate flow, a water bath (37°C), and a fraction collector. The perifusion solution was Krebs buffer (pH 7.4) containing 2.2 mmol/L Ca^{2+}, 0.25%

of BSA, 0.5% of DMSO, 5 mmol/L of ketoisocaproic acid, and equilibrated with 95% O_2 and 5% CO_2. Insulin in efflux samples was evaluated using RIA procedure [21]. A total of 100 islets were used in every single perifusion experiment. Islets were preperifusion with no substrate added for 38 minutes followed either by glyburide, *N*-{4-[β-(2-(2′-fluoroethoxy)-5-iodobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (8a) or *N*-{4-[β-(2-(2′-fluoroethoxy)-5-chlorobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (8b) RAMP (50 nmol/L/min up to 2 μmol/L) for another 40 minutes. After washing substrate out, maximal insulin release was tested with 30 mmol/L of KCl.

2.7. In vitro binding studies

The *in vitro* binding of F-18 labeled compound 8a or 8b to two mouse pancreatic β cell lines, βTC3 and Min6 cells was carried out in triplicate. The βTC3 cells were maintained in RPMI 1640 supplemented with 15% fetal bovine serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L L-glutamine at 37°C in a humidified 5% CO_2 incubator. Min6 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L L-glutamine at 37°C in a humidified 5% CO_2 incubator. The cells were grown in six-well plate as monolayer with 70-90% confluence. For the total binding, F-18 labeled compound 8a or 8b in 40 μL of saline was added to each well containing either 1.3×10^6 βTC3 cells or 0.5×10^6 Min6 cells in 1 mL of culture medium. The plate was incubated at 25°C for 60 minutes, the supernatant was removed by aspiration, and the wells were washed with PBS (4 mL × 2). The cells were harvested with trypsin (2 mL/well). The harvested cells and the combined supernatants were counted in a NaI gamma well counter (Packard series 5000). The percent of total binding was calculated as the radioactivity in the harvested cells divided by the combined radioactivity in the harvested cells and the combined supernatants. For the specific binding, 0, 1, 10, 100, 1000, and 10,000 nmol/L of nonradioactive compound 8a or 8b was added to the well containing either βTC3 or Min6 cells and F-18 labeled compound 8a or 8b. The mixture was incubated at 25°C for 60 minutes and processed as described above.

2.8. Biodistribution in mice

Both normal and streptozotocin (20 mg/kg, twice)–induced diabetic SCID mice weighing 27-35 g were used for each biodistribution study. A quantity of 15-20 μCi of either compound 8a or compound 8b in 0.1 mL of saline solution was injected into the tail vein. The mice were killed by cervical dislocation at 30, 60, and 120 minutes postinjection. Organs of interest were removed, blotted to remove adhering blood, and placed in tared counting vials. The radioactivity of each sample was measured in a NaI well counter and the sample weighed. The percentage dose per gram of tissue was calculated by a comparison of the tissue radioactivity to 100 times diluted aliquots of the injected tracer measured at the same time.

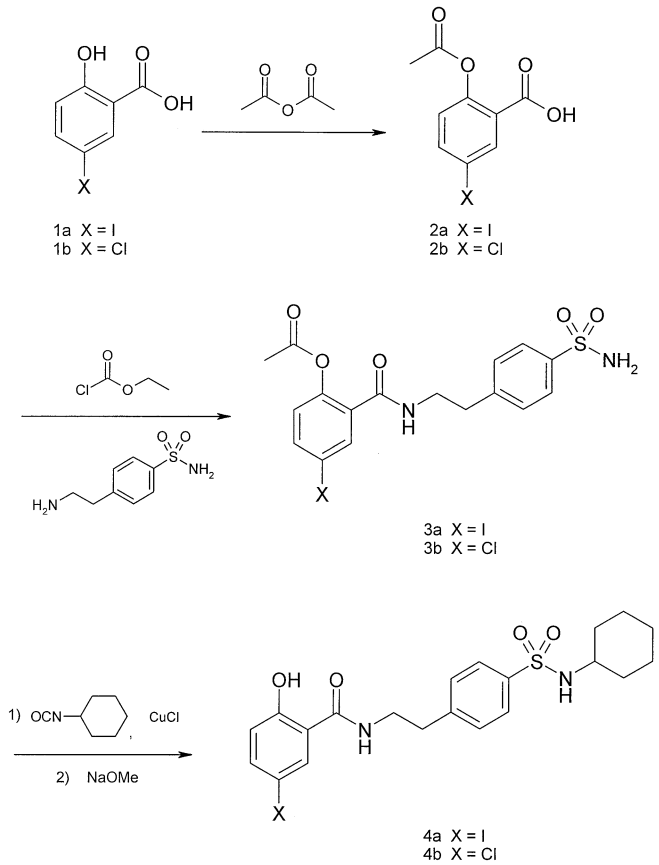
For blocking experiment, the mice were pretreated with nonradioactive compound 8a (2 mg/kg) 30 minutes before intravenous injection of radioactive compound 8a into the tail vein. The mice were killed by cervical dislocation at 60 minutes postinjection and processed as described above.

3. Results and discussion

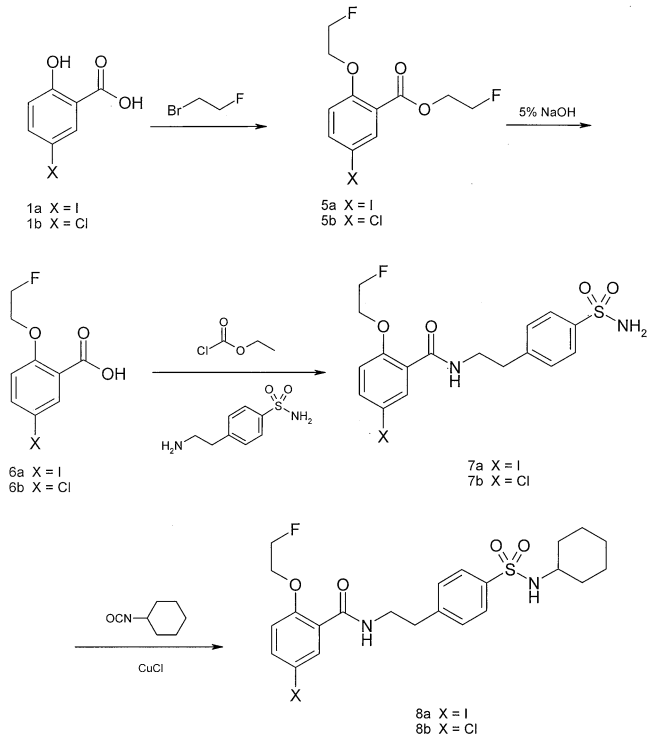
To date, there have been no reported techniques to image the endocrine pancreas. Recently, several radioactive compounds, such as ^{65}Zn [22], [^{3}H]mitiglinide [23], a ^{125}I-labeled mouse monoclonal antibody directed against pancreatic β-cell surface ganglioside(s) [24], and a ^{111}In labeled monoclonal antibody specific for mouse pancreatic β-cells [25] have been used to visualize β-cells *in vitro* or *in vivo*, but none of them was successful or feasible for clinical application in humans. Glyburide, a widely prescribed drug to treat type 2 diabetes, has high binding affinity to SUR and the binding is saturable *in vitro* [14]. These characteristics strongly suggested that positron emitter–labeled glyburide or its analogs may serve as β-cell imaging agents. We have synthesized two of its analogs (8a, 8b) and evaluated them *in vitro* and *in vivo* as β-cell imaging agents.

Both *N*-{4-[β-(2-hydroxy-5-chlorobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (4b) and *N*-{4-[β-(2-hydroxy-5-iodobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (4a) were prepared from the corresponding salicylic acid in a multistep synthesis as reported previously [18,20] (Scheme 1). Reaction of 5-iodosalicylic acid (1a) with acetic anhydride gave 2-acetoxy-5-iodobenzoic acid (2a). Reaction of compound 2a with ethyl chloroformate followed by *p*-(β-aminoethyl)benzenesulfonamide gave 4-[β-(2-acetoxy-5-iodobenzenecarboxamido)ethyl]benzenesulfonamide (3a). Reaction of compound 3a with cyclohexyl isocyanate gave *N*-{4-[β-(2-hydroxy-5-iodobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (4a).

N-{4-[β-(2-(2′- fluoroethoxy)-5-iodobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (8a) was prepared from either a multistep synthesis as that described for the synthesis of *N*-{4-[β-(2-(2′-fluoroethoxy)-5-chlorobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (8b) [18] or from a one-step alkylation of the multistep synthesized compound 4a with 1-bromo-2-fluoroethane (Scheme 2). Reaction of 5-iodosalicyclic (1a) with 1-bromo-2-fluoroethane and sodium hydride gave 2′-fluoroethyl 2-(2′-fluoroethoxy)-5-iodobenzoate (5a). Deprotection of compound 5a with 5% NaOH solution gave 2-(2′-fluoroethoxy)-5-iodobenzoic acid (6a). Reaction of compound 6a with ethyl chloroformate followed by *p*-(β-



Scheme 1. Synthesis of precursors.



Scheme 2. Synthesis of 2-fluoroethoxy-5-iodoglyburide (**8a**) and 2-fluoroethoxyglyburide (**8b**).

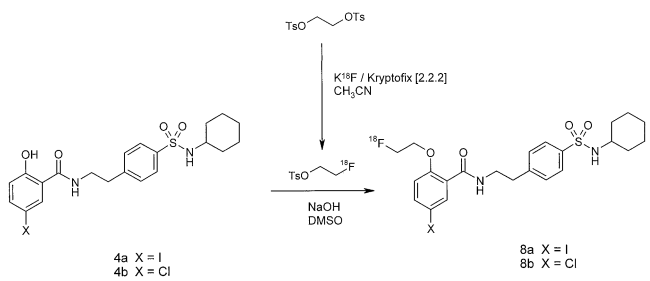
aminoethyl)benzenesulfonamide gave 2-(2′-fluoroethoxy)-5-iodobenzenecarboxamido)ethyl]benzenesulfonamide (7a). Reaction of sulfonamide (7a) with cyclohexyl isocyanate gave *N*-{4-[*β*-(2-(2′-fluoroethoxy)-5-iodobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (8a) in an overall yield of ~10%. The identities of all of these precursors and authentic samples were verified by NMR or elemental analysis.

Direct alkylation of compound 4a with 1-bromo-2-fluoroethane at 60°C for 3 days gave compound 8a in 6% yield. Thus, a multistep synthesis is probably a better method to prepare this product.

Fluorine-18 labeled *N*-{4-[*β*-(2-(2′-fluoroethoxy)-5-iodobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (8a) was synthesized in two steps (Scheme 3). Nucleophilic substitution of ethylene glycol di-*p*-tosylate with K[^{18}F]/Kryptofix$_{2.2.2}$ gave 1-[^{18}F]fluoro-2-tosylethane in 50% yield. Alkylation of *N*-{4-[*β*-(2-hydroxy-5-iodobenzene carboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (4a) with 1-[^{18}F]fluoro-2-tosylethane followed by HPLC purification gave F-18 labeled compound 8a in an overall yield of 5-10% yield in a synthesis time of 100 minutes from EOB. The specific activity was 0.5 Ci/μmol. The identity of F-18 labeled compound 8a was verified by both TLC and HPLC compared to the nonradioactive authentic sample. Fluorine-18 labeled compound 8b was synthesized as reported previously [18].

The partition coefficients of compound 8a and compound 8b were 141.21 ± 27.77 (n = 8) and 124.33 ± 21.61 (n = 8), respectively. The difference in lipophilicity of these two compounds was also evident from their retention times in a C_{18} column. The retention times of compound 8a and compound 8b in HPLC (Phenomenex, Luna 2, C_{18}, 4.6 × 250 mm, CH_3OH:H_2O, 7:3; 1 mL/min) were 17.6 and 14.2 minutes, respectively. Although there was not a great difference in binding affinity to sulfonylurea receptor, the difference in lipophilicity of compounds 8a and 8b may affect their usefulness as *β*-cell imaging agents.

The feasibility of using compounds 8a and 8b as *β*-cell imaging agents was evaluated preliminary with their binding to whole *β*-cells, their abilities to stimulate insulin release from rat islets, and their biodistribution in both



Scheme 3. Synthesis of F-18 labeled 2-fluoroethoxy-5-deschloro-5-iodoglyburide (**8a**) and 2-fluoroethoxyglyburide (**8b**).

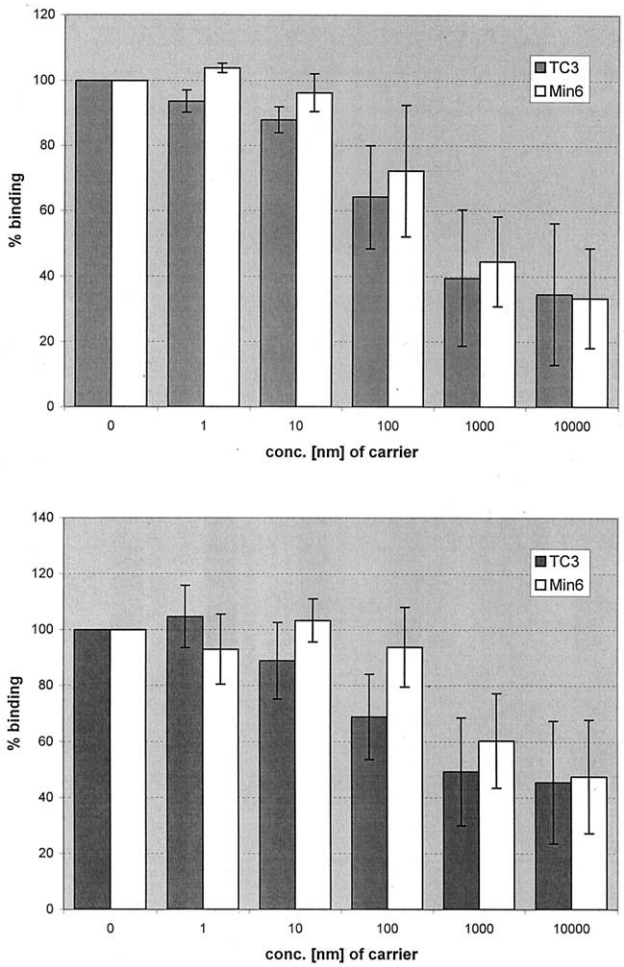


Fig. 2. *In vitro* binding of F-18 labeled compounds **8a** (top panel) and **8b** (bottom panel) to βTC3 (filled squares) and Min6 cells (open squares) with and without carrier added. The results show that the binding is saturable.

normal and diabetic mice. The *in vitro* binding of F-18 labeled compound 8a and 8b to whole βTC3 and Min6 cells were similar (~2%). Figure 2 shows that the binding was inhibited by co-incubation with nonradioactive compound 8a, 8b, suggesting that the *in vitro* binding of F-18 labeled compound 8a and 8b was probably receptor mediated. Figure 3 shows that compounds 8a and 8b have a similar ability as glyburide itself to stimulate the release of insulin from rat islets. These preliminary *in vitro* results tend to suggest that compounds 8a and 8b may be useful for β-cell imaging. However, *in vivo* studies in both the normal and diabetic mice showed that the uptake of compound 8b in liver and intestine were high, but its uptake in pancreas was low (Fig. 4). Additionally, pretreatment of compound 8a with nonradioactive compound 8a did not significantly block the uptake of compound 8a in the pancreas (Fig. 5), suggesting that the uptake of compound 8a in the pancreas was probably not SUR mediated. A recent study with [^{11}C]glyburide also independently showed the uptake of [^{11}C]glyburide in rat liver is high and nonspecific [26]. Thus, compound 8a, 8b, and [^{11}C]glyburide may not be the ideal tracers for

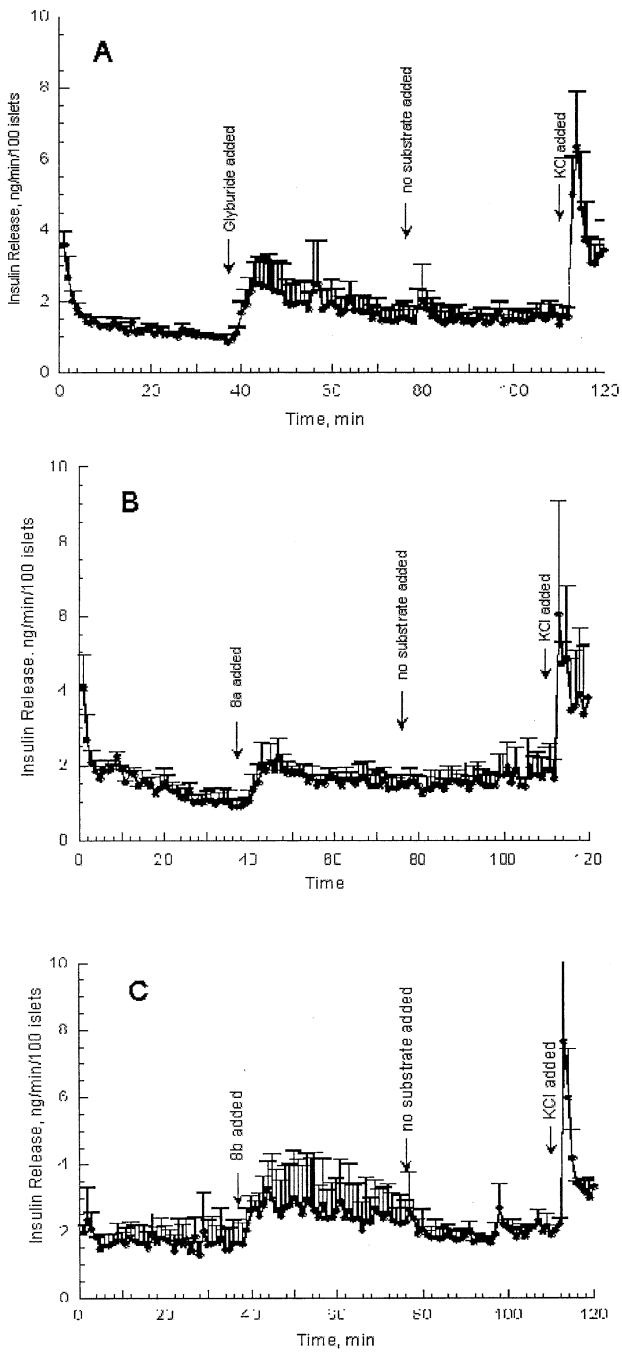


Fig. 3. Effects of glyburide (panel A) and compounds 8a (panel B) and **8b** (panel C) on insulin secretion from rat islets. The results show that both compounds **8a** and **8b** are insulin secretagogues.

β-cell imaging. The reasons for this are not clear. However, as both [^{11}C]glyburide and compound 8b have different specific activity (1.5 and 0.5 Ci/μmol, respectively) but similar biodistribution patterns, the relatively low specific activity of compound 8b may not be the main reason that glyburide is not the ideal tracer for β-cell imaging. On the other hand, internalization of SUR [27,28], high lipophilicity, and similar binding affinity to both SUR1 and SUR2 (K_{ds} for SUR1 and SUR2 are 14.4 nmol/L and 25 nmol/L,

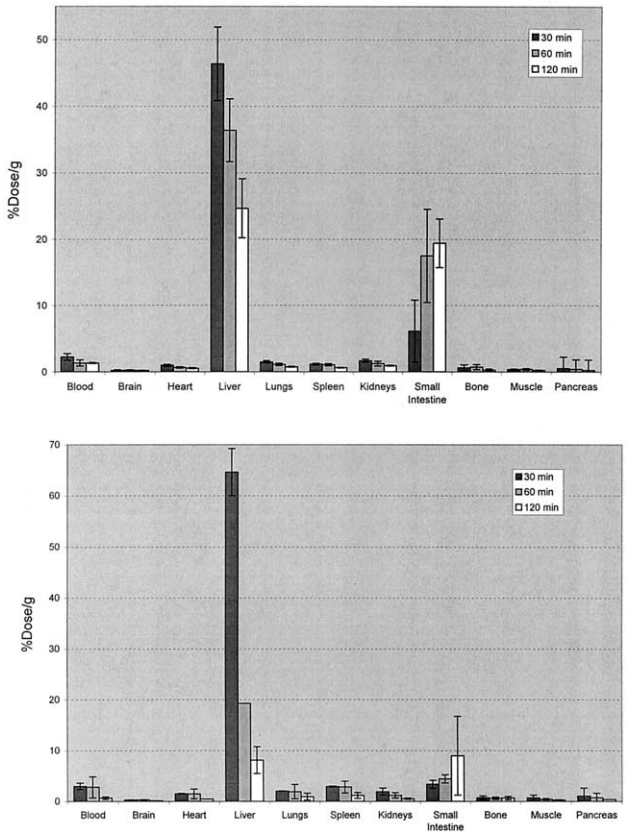


Fig. 4. Biodistribution of F-18 labeled compound **8b** in normal (top panel) and diabetic (bottom panel) SCID mice ($n = 3$). The results show that the uptake pattern is similar between normal and diabetic mice, and that the uptake of **8b** in liver and small intestine are high, whereas its uptake in pancreas is low.

respectively) [29] may be the reasons for glyburide being not the ideal tracer for β-cell imaging. SUR1 density is high in endocrine pancreas (β-cells), whereas SUR2 density is high in cardiac, skeletal, and smooth muscle [30-32]. Because glyburide has similar binding affinity to both SUR1 and SUR2, it will have high uptake in other tissues besides those of the endocrine pancreas and therefore will increase

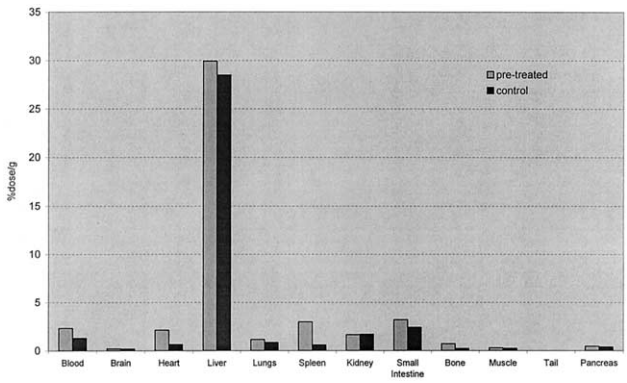


Fig. 5. Effects of carrier on the biodistribution of F-18 labeled compound **8a** in mice ($n = 3$). The results show that the uptake of F-18 labeled compound **8a** in mice is nonsaturable.

background activity. The search for ligands that are less lipophilic than glyburide and are more selective to SUR1 continues.

Taken collectively, we have synthesized two fluorinated glyburide analogs, namely *N*-{4-[β-(2-(2′-fluoroethoxy)-5-iodobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (2-fluoroethoxy-5-deschloro-5-iodoglyburide, 8a) and *N*-{4-[β-(2-(2′-fluoroethoxy)-5-chlorobenzenecarboxamido)ethyl]benzenesulfonyl}-*N*′-cyclohexylurea (2-fluoroethoxyglyburide, 8b), and evaluated them as β-cell imaging agents. Although the *in vitro* studies showed that both compounds 8a and 8b may be the tracers for β-cell imaging, their high uptake in the liver and intestine, as well as the low and nonspecific uptake in the pancreas, suggested that both compounds 8a and 8b may not be the ideal tracers for β-cell imaging. However, because of its rapid washout from the liver, compound 8a labeled with longer half-life radionuclide (I-123 or I-124) may serve as β-cell imaging agent.

Acknowledgments

The authors thank Mr. Harry J. White for providing [^{18}F]fluoride. This work was partially supported by a pilot grant from the Society of Nuclear Medicine (C-Y S) and Juvenile Diabetes Foundation International (Ali Naji).

References

[1] Taylor SI. Deconstructing type 2 diabetes. Cell 1999;97:9–12.
[2] DeFronzo R Pathogenesis of type-2 diabetes—metabolic and molecular implications for identifying diabetes genes. Diabetes Rev1997; 5:177-269.
[3] Chehade JM, Mooradian AD. A rational approach to drug therapy of type 2 diabetes mellitus. Drugs 2000;60:95–113.
[4] Bottino R, Lemarchand P, Trucco M, Giannoukakis N. Gene- and cell-based therapeutics for type I diabetes mellitus. Gene Ther 2003; 10:875–89.
[5] Uchikoshi F, Yang ZD, Rostami S, Yokoi Y, Capocci P, Barker CF, Naji A. Prevention of autoimmune recurrence and rejection by adenovirus-mediated CTLA4Ig gene transfer to the pancreatic graft in BB rat. Diabetes 1999;48:652–7.
[6] Norrchashm H, Noorchashm N, Kern J, Rostami SY, Barker CF, Naji A. B-cells are required for the initiation of insulitis and sialitis in nonobese diabetic mice. Diabetes 1997;46:941–6.
[7] Edwards G, Weston A. The pharmacology of ATP-sensitive potassium channels. Annu Rev Pharmacol Toxicol 1993;33:597–637.
[8] Proks P, Reimann F, Green N, Gribble F, Ashcroft F. Sulfonylurea stimulation of insulin secretion. Diabetes 2002;51(suppl 3):368–76.
[9] Ashcroft SJH, Niki I, Kenna S, Weng L, Skeer L, Coles B, Ashcroft FM. The β-cell sulfonylurea receptor. Adv Exp Med Biol. 1993;334: 47−61.
[10] Panten U, Schwanstecher M, Schwanstecher C. Pancreatic and extrapancreatic sulfonylurea receptors. Horm Metab Res 1992;24:549–54.
[11] Meyer M, Chudziak F, Schwanstecher C, Schwanstecher M, Panten U. Structural requirements of sulphonylureas and analogues for interaction with sulphonylurea receptor subtypes. Br J Pharmacol 1999; 128:27–34.

[12] Niki I, Welsh M, Berggren PO, Hubbard P, Ashcroft SJ. Characterization of the solubilized glibenclamide receptor in a hamster pancreatic beta-cell line, HIT T15. Biochem J 1991;277:619–24.
[13] Gaines KL, Hamilton S, Boyd AE III. Characterization of the sulfonylurea receptor on beta cell membranes. J Biol Chem 1988;263: 2589–92.
[14] Schmid-Antomarchi H, De Weille J, Fosset M, Lazdunski M. The receptor for antidiabetic sulfonylureas controls the activity of the ATP-modulated K+ channel in insulin-secreting cells. J Biol Chem 1987;262:15840–4.
[15] Aguilar-Bryan L, Nichols CG, Rajan AS, Parker C, Bryan J. Coexpression of sulfonylurea receptors and KATP channels in hamster insulinoma tumor (HIT) cells. Evidence for direct association of the receptor with the channel. J Biol Chem 1992;267:14934–40.
[16] Hokfelt B, Jonsson A. Hypoglycemic activity in relation to chemical structure of potential oral antidiabetic substances. I. 1-Sulfonyl-3-alkylureas. J Med Pharm Chem 1962;5:231–9.
[17] Schirrmacher R, Weber M, Schmitz A, Shiue C-Y, Alavi AA, Feilen P, Schneider S, Kann P, Rösch F. Radiosynthesis of 1-(4-(2-[18F]Fluoroethoxy)benzenesulfonyl)-3-butyl urea: a potential β-cell imaging agent. J Label Compd Radiopharm 2002;45:763–74.
[18] Shiue GG, Schirrmacher R, Shiue C-Y, Alavi AA. Synthesis of fluorine-18 labeled sulfonureas as β-cell imaging agents. J Label Compd Radiopharm 2001;44:127–39.
[19] Shiue C-Y, Schmitz A, Pourdehnad MT, Shiue GG, Vatamaniuk M, Doliba N, Matschinsky F, Wolf B, Alavi AA, Schirrmacher R. Synthesis of β-cell imaging agents. J Nucl Med 2001;42(suppl): 72P.
[20] Aguilar-Bryan L, Nelson DA, Vu QA, Humphrey MB, Boyd AE III. Photoaffinity labeling and partial purification of the β cell sulfonylurea receptor using a novel, biologically active glyburide analog. J Biol Chem 1990;265:8218–24.
[21] Herbert V, Lau KS, Gottlieb CW, Bleicher S. Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab 1965;25:1375–84.
[22] Ladriere L, Malaisse-Lagae F, Malaisse WJ. Pancreatic uptake of 65zinc in control and streptozotocin injected rats. Med Sci Res 2000; 28:43–4.
[23] Malaisse WJ. On the track to the beta-cell. Diabetologia 2001;44: 393–406.
[24] Ladriere L, Malaisse-Lagae F, Alejandro R, Malaisse WJ. Pancreatic fate of a 125I-labeled mouse monoclonal antibody directed against pancreatic B-cell surface ganglioside(s) in control and diabetic rats. Cell Biochem Funct 2001;19:107–15.
[25] Moore A, Bonner-Weir S, Weissleder R. Noninvasive in vivo measurement of beta-cell mass in mouse model of diabetes. Diabetes 2001;50:2231–6.
[26] Hwang DR, Pham V, Huang Y, Laruelle M, Jerabek P, Fox P, DeFranzo R. Synthesis and in vivo evaluation of [11C]glyburide for imaging pancreatic β-cells. J Label Compd Radiopharm 2003;46: S352.
[27] Carpentier J-L, Sawano F, Ravazzola M, Malaisse WJ. Internalization of 3H-glibenclamide in pancreatic islet cells. Diabetologia 1986;29: 259–61.
[28] Ladriere L, Malaisse-Lagae F, Malaisse WJ. Uptake of tritiated glibenclamide by endocrine and exocrine pancreas. Endocrine 2000;13: 133–6.
[29] Fuhlendorff J, Rorsman P, Kofod H, Brand CL, Rolin B, MacKay P, Shymko R, Carr RD. Stimulation of insulin release by repaglinide and glibenclamide involves both common and distinct processes. Diabetes 1998;47:345–51.
[30] Suzuki M, Fujikura K, Kotake K, Inagaki N, Seino S, Takata K. Immuno-localization of sulphonylurea receptor 1 in rat pancreas. Diabetologia 1999;42:1204–11.
[31] Suzuki M, Fujikura K, Inagaki N, Seino S, Takata K. Localization of the ATP-sensitive K+ channel subunit Kir6.2 in mouse pancreas. Diabetes 1997;46:1440–4.
[32] Chutkow WA, Simon MC, Le Beau MM, Burant CF. Cloning, tissue expression, and chromosomal localization of SUR2, the putative drug-binding subunit of cardiac, skeletal muscle, and vascular K^{ATP} channels. Diabetes 1996;45:1439–45.