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Synthesis and evaluation of radioiodinated substituted β-naphthylalanine as a potential probe for pancreatic β-cells imaging

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Abstract

A non-invasive imaging technique capable of relating a signal from the β-cells to their mass will be of immense value in understanding the progression of diabetes.

Several molecular markers have indeed been identified and investigations are ongoing aimed at accomplishing the said goal. These include pancreatic islet antigen (IC-2), somatostatin receptors (SSTRs), and sulfonylurea receptors (SURs) on the pancreatic β-cells. Therefore investigations exploiting the potential application of the radiolabeled ligands for these receptors for β-cell imaging are receiving intensive research attention. Radioiodinated peptidomimetic based on β-naphthylalanine and *n*-hexanediamine has been synthesized. The molecule was subjected to in vitro and in vivo evaluation. Radioligand binding studies on CHO cell line expressing the SSTR2 showed very low affinity. Nonetheless, biodistribution in normal mice showed significant uptake in the pancreas. There was partial blockage of the pancreatic uptake when excess of the peptidomimetic was coinjected. The result implies that the pancreatic uptake was receptor mediated but may not involve the SSTR2 and therefore warrants further investigation.

Keywords: Diabetes; Peptidomimetics; Somatostatin receptors; Non-invasive imaging; β-cell mass

1. Introduction

Insulin-dependent diabetes mellitus (IDDM, type-1) is characterized by an autoimmune process leading to the destruction of β-cells in individuals who are genetically predisposed to the disease (Atkinson and Maclaren, 1994; Todd, 1995; Verge et al., 1996; Srikanta, et al., 1984). Several hypotheses have been proposed to explain the progressive destruction of β-cells. One such theory was that the destructive process progresses at a linear rate from the initial trigger to the final appearance of symptoms. Another proposed explanation was that there was a multi-hit pattern of repeated exacerbation that eventually leads to destruction of the β-cells. However, it is clear that one hallmark of the disease is the infiltration of the pancreas by mononuclear cells that begins long before the onset of the disease and progressively decreases as the β-cell mass gradually declined (Skyler, 2004; Kahn, 2003; Rossini et al., 1993; Pipeleers and Ling, 1992). Consequently after sufficient loss of β-cell mass and function, invariably there is the need for therapeutic insulin replacement.

Type 2 diabetes (the common type) is usually due to insulin resistance in a setting of inadequate compensatory insulin secretory response. Current treatment approaches for type 2 diabetes include diet, exercise, and variety of pharmacological intervention agents including insulin, biguanides, sulfonylureas (SUs), and thiazolidinediones (Skyler, 2004). These agents all act by different mechanisms in an attempt to normalize blood glucose level and to prevent all the recognized serious complications of diabetes, such as renal failure, cardiovascular, ophthalmic diseases, etc.

The success of these interventional strategies may depend largely on a clear understanding of the disease progression. It is known that the residual β-cell mass at diagnosis is an important factor in the success of any

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treatment (Pozzilli and Maclaren, 1993). The lack of a technique to non-invasively measure or visualize pancreatic β-cells has left many unanswered questions regarding the natural history of disease progression. There is therefore an urgent need for a non-invasive imaging method(s) to provide answers to these crucial but outstanding questions pertaining to diabetes.

Nuclear imaging is one of the non-invasive imaging modalities that have seen tremendous progress over the last two decades in comparison with other imaging methods (Weissleder, 1999). The new trends in the development of tracers for positron emission tomography (PET) and single-photon emission tomography (SPECT) are based on biochemical concepts. In this regard natural substrates and drug molecules are labeled with short-lived organic positron or single-photon emitting radionuclides.

In-111-labeled autologous lymphocytes were used to detect pancreatic-infiltrating lymphocytes in newly diagnosed patients with type 1 diabetes, but the study was only partially successful (Kaldany et al., 1982). Signore and coworkers reported the use of I-123-Interleukin-2 to assess auto immunity and diabetes (Signore et al., 1992, 1987). Interleukin-2 has also been labeled with Tc-99m intended for the same purpose; however, this method also measured the extent of mononuclear cell infiltration in immune-mediated diseases in general including type 1 diabetes (Chianelli et al., 1997).

A concerted effort made to image type 1 diabetes and to establish the prognostic relevance of technetium-99m-labeled human polyclonal immunoglobulins (Tc-99m-HIG) in human diabetic patients has been reported (Barone et al., 1998). Recently, a specific antibody to the islet antigen was used to measure the beta cell mass in both in vitro and in vivo in mice (Moore et al., 2001; Brogren et al., 1986; Aaen et al., 1990). Modulations of insulin secretion by antidiabetic secretagogues involve the binding to the high-affinity SU receptors (SURs) expressed by the β-cells. Hence several of the SU and analogs have been radiolabeled and investigated as potential nuclear imaging agents. These molecules have been labeled with PET radionuclides such as F-18 and C-11 (Schmitz et al., 2004; Wängler et al., 2004; Hwang et al., 2003).

Natural somatostatin is a cyclic tetradecapeptide (SST-14) and SST-28, a congener of SS-14, produced by and present in the hypothalamus, adrenals and the pancreas. The actions of somatostatin are mediated through specific membrane receptors. So far five sub-types of human somatostatin receptors (hSSTR1-5) have been cloned and characterized. The five receptor subtypes bind SST-14 and SST-28 with low nanomolar (nM) affinity. Several SST peptide analogs have been synthesized and developed that are long acting and stable in vivo. Reports on the selectivity of all five subtypes for these synthetic analogs have been controversial (Reubi et al., 1990; Bruns et al., 1996; Raynor et al., 1993; Patel and Srikant, 1994). Nonetheless, several systematic studies on analogs of SS have produced peptides selective for the subtypes (Weckbecker et al., 2003; Erchegyi et al., 2005).

It has been established that in the pancreas (mouse, rat and human) insulin secretion is associated with the β-cells. Additionally SSTR1 and to approximately the same extent SSTR5 have been co-localized on these cells (Moldovan et al., 1995; Fagan et al., 1998; Mitra et al., 1999; Reubi et al., 1998; Kumar et al., 1999).

Recently, several peptidomimetics that show subtype selectivity was reported (Rohrer et al., 1998). These are synthetic non-peptide molecules designed to retain the receptor recognition (pharmacophores) components of SST. These molecules were shown to display affinity for the SSTRs in the picomolar to low nanomolar range. Some of these molecules modulate insulin secretion. It is hypothesize that radiolabeled analogs of these non-peptide molecules may serve as radioligands for β-cells. This paper reports the synthesis, radioiodination and evaluation of a substituted naphthylalanine derivative as a potential pancreatic β-cells imaging agent.

2. Materials and methods

All the general chemicals and reagents were purchased from Sigma-Aldrich, Fisher or Fluka. The chemicals were used as such without further purification except where indicated. Iodine ^{125}I and ^{131}I were purchased from Amersham Inc. UK and The Institute of Isotopes, Budapest, Hungary, respectively. ESI-MS was run on a Quattro system. The ^{1}H-NMR was acquired on a Beckman 400 MHz spectrometer using TMS as the reference and the chemical shifts are recorded in ppm.

The high-performance liquid chromatography (HPLC) analysis was carried out on Econosil C-18, 10 μm columns (semi-preparative, 250 mm × 10 mm or analytical, 250 mm × 4.6 mm). A gradient elution method was used with solvent $A = 0.1\%$ TFA in water and $B = 0.1\%$ TFA in acetonitrile. The program was as follows: start 100% A; 5 min, 90%; 10 min, 10%; 25 min, 10% and 30 min, 0%. The flow rates were 4.0 mL/min and 1 mL/min for the semi-preparative and analytical columns, respectively. A JASCO (Tokyo, Japan) chromatographic system equipped with a variable wavelength ultraviolet monitor and in tandem with a Canberra flow through radioactivity detector was used. Ultraviolet absorption was monitored at 254 nm. Chromatograms were acquired and analyzed using BOR-WIN® software. Student's *t*-test was used for statistical analysis, *P*-value less than 0.05 was considered significant.

2.1. Synthesis of tert-butyl-6-amino-3,5,5-trimethylhexylcarbamate (2)

2,2,4/2,4,4-Trimethylhexanediamine (**1**) (5 mL, 27.4 mmol) was dissolved in cold chloroform (100 mL) (Scheme 1). The di-tert-butyl dicarbonate (1.2 g, 5.5 mmol) was also dissolved in chloroform (50 mL) and placed in a dropping funnel. This was added dropwise to the diamine solution while stirring,