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Altered serotonin (5-HT) 1D and 2A receptor expression may contribute to defective insulin and glucagon secretion in human type 2 diabetes

H. Bennet[a], A. Balhuizen[a], A. Medina[a], M. Dekker Nitert[a,b], E. Ottosson Laakso[a], S. Essén[c], P. Spégel[a], P. Storm[a], U. Krus[a], N. Wierup[a], M. Fex[a,*]

[a] Department of Clinical Science, Lund University Diabetes Centre, Scania University Hospital, Entrance 72, Clinical Research Centre House 91, Jan Waldenströmsgata 35, SE-20502, Malmö, Sweden
[b] Royal Brisbane Clinical School, UQ Centre for Clinical Research, The University of Queensland, Herston, QLD 4029, Australia
[c] The Centre for Analysis and Synthesis, Department of Chemistry, Lund University, Getingevägen 60, SE-22241, Lund, Sweden

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ABSTRACT

Islet produced 5-hydroxy tryptamine (5-HT) is suggested to regulate islet hormone secretion in a paracrine and autocrine manner in rodents. Hitherto, no studies demonstrate a role for this amine in human islet function, nor is it known if 5-HT signaling is involved in the development of beta cell dysfunction in type 2 diabetes (T2D). To clarify this, we performed a complete transcriptional mapping of 5-HT receptors and processing enzymes in human islets and investigated differential expression of these genes in non-diabetic and T2D human islet donors. We show the expression of fourteen 5-HT receptors as well as processing enzymes involved in the biosynthesis of 5-HT at the mRNA level in human islets. Two 5-HT receptors (HTR1D and HTR2A) were over-expressed in T2D islet donors. Both receptors (5-HT1d and 5-HT2a) were localized to human alpha, beta and delta cells. 5-HT inhibited both insulin and glucagon secretion in non-diabetic islet donors. In islets isolated from T2D donors the amine significantly increased release of insulin in response to glucose. Our results suggest that 5-HT signaling participates in regulation of overall islet hormone secretion in non- diabetic individuals and over-expression of HTR1D and HTR2A may either contribute to islet dysfunction in T2D or arise as a consequence of an already dysfunctional islet.

1. Introduction

Type 2 diabetes (T2D) is a heterogeneous disorder caused by a combination of insulin resistance and insufficient insulin secretion [1] . Regulation of overall islet hormone secretion is achieved by an intricate interplay between metabolic and nervous input to the pancreatic islet cells. Failure of such signals to the beta cells may result in overt T2D.

Monoaminergic neurotransmitters stored within the islets have been proposed to regulate glucose stimulated insulin secretion (GSIS) in a paracrine and/or autocrine fashion in rodents [2,3]. This circuitry of monoamines most likely receives input from sympathetic and parasympathetic innervation of islets, which at least in rodents play an important role in regulation of hormone secretion as rodent islets are richly supplied with both cholinergic and adrenergic axons [4]. In humans however, islets appear more autonomous from central control, as islets are sparsely innervated, rather axons are associated with smooth muscle cells of blood vessels within the islets [5]. Thus, in human islets, intra islet signaling mediated by monoamines may play a more pronounced role in the control of overall islet hormone secretion.

Serotonin (5-hydroxy tryptamine (5-HT)) is a neurotransmitter in the central, and in the peripheral nervous systems, and a hormone produced by enterochromaffin cells in the gut [6]. 5-HT is synthesized from the essential amino acid tryptophan, involving the enzymatic activity of tryptophan hydroxylase (TPH1 and TPH2) and dopa decarboxylase (DDC). The amine acts on target cells through receptors encoded by 17 genes and numerous splice variants [7], or by covalent binding to small GTPases [8]. 5-HT is present in islets of several mammalian species [9,10] indicating a role for this amine in islet cell function and recent studies show that

* Corresponding author at: Clinical Research Centre, House 91:10, Jan Waldenströmsgata 35, SE-20502, Malmö, Sweden. Fax: +46 40391212.
E-mail address: malin.fex@med.lu.se (M. Fex).

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murine islet express both TPH1 and TPH2 [11,12], suggesting production of 5-HT. Previous studies have shown conflicting results of the effects of 5-HT on insulin secretion [8,13,14] and diabetogenic effects of atypical antipsychotics and antidepressants urges an increased understanding of peripheral effects of 5-HT modulating therapies upon glucose homeostasis [15].

Virtually all 5-HT receptors act through G-proteins, activating either G_i, G_s or G_q, except for the 5-HT3 family, comprising ligand gated ion channels primarily for K^+ and Na^+ [7] . The 5-HT receptors 5-HT1a, 5-HT1d, 5-HT2b and 5-HT3a/b are present in rodent islets [12,16,17] and expression of 5-HT1a [18], 5-HT2a, 2b and 2c [19] receptors has been reported in the human pancreas. Others report only trace amounts (at the mRNA level) of the *HTR1A*, *HTR1B* and *HTR1D* receptor in human islets, while expression of the *HTR1F* receptor is abundant [20].

Several studies advocate an important role for 5-HT signaling in the endocrine pancreas during pregnancy. Production of 5-HT is increased in islets from pregnant rodents [11,12] and the 5-HT2b receptor regulates the compensatory increase of beta cell mass, while the 5-HT1d receptors mediate decrease of beta cell mass, post partum [12]. Moreover, the 5-HT3 receptor family is suggested to be necessary to induce compensatory increase of insulin secretion during pregnancy [17] . However, there is currently no known role for 5-HT in human islets and there is no proof of the involvement of 5-HT signaling in islet dysfunction and T2D. Therefore, we aimed to perform a complete transcriptional mapping of 5-HT receptors in human diabetic and non-diabetic islets. We also aimed to determine islet cellular localization and the role for selected receptors in human islet function.

2. Material and methods

2.1. Human donor material

Islets from human donors were obtained from the Nordic Network for Clinical Islet Transplantation in Uppsala, Sweden, via the Human Tissue Laboratory at Lund University Diabetes Center, Malmö, Sweden. In Uppsala, human islets were isolated using a modification of the semi-automated digestion–filtration method as previously described [21] and, followed by purification on a continuous density gradient in a refrigerated COBE 2991 centrifuge (COBE Blood Component Technology, Lakewood, CO). The islet preparations were placed in untreated petri dishes Sterilin (Tamro Med., Lab., AB) and kept at 37 °C in an atmosphere of 5% CO_2 in humidified air in culture medium, CMRL 1066 (Gibco-BRL, Invitrogen) supplemented with 10 mM nicotinamide (Sigma Chemicals), 10 mM Hepes buffer (Gibco-BRL, Invitrogen), 0.25 μg/ml fungizone (Gibco-BRL, Invitrogen), 50 μg/ml gentamicin (Gibco-BRL, Invitrogen), 2 mM L-gLutamine (Gibco-BRL, Invitrogen), 10 μg/ml ciprofloxacin (Bayer), and 10% (v/v) heat-inactivated human serum. The islets were cultured for 2–5 days and medium was changed every second day before arrival to Lund University Diabetes Center in Malmö, Sweden, where experiments were performed the following day. The ethics committees in Uppsala and Lund, Sweden, approved the studies. All chemicals were purchased from Sigma Aldrich, Sweden, unless otherwise stated.

2.2. RNA sequencing

Total RNA was extracted from 131 human islet donors, and RNA-seq libraries were generated (TruSeq RNA sample preparation kit, Illumina) and sequenced on an Illumina HiSeq 2000 using paired-end chemistry and 100 bp cycles to an average depth of 32 M read pairs per sample. Reads were aligned to hg19 using STAR (http://www.ncbi.nlm.nih.gov/pubmed/23104886) and reads were summarized by feature Counts (bioinformatics.oxfordjournals.org/content/early/2013/11/13/bioinformatics.btt656.abstract) using Gencode gene annotations version 19 (www.gencodegenes.org) and normalized using trimmed mean of *M* values. Differential expression was assessed by linear regression using the R-package limma [22].

2.3. Quantitative PCR in human islets of Langerhans

mRNA was prepared from human islets (RNAeasy mini kit, Qiagen, Valencia, CA, USA) and cDNA obtained by reverse transcription (Maxima™ First Strand cDNA Synthesis Kit, Fermentas, Thermo Scientific, Helsingborg, Sweden). mRNA levels were quantified using a Probe/Rox Real-Time PCR (Maxima™ Probe/ROX qPCR Master Mix (2X), Fermentas, Thermo Scientific, Helsingborg, Sweden) with an ABI PRISM 7900 (Applied Biosystems, Foster City, CA, USA). Assays-on-demand for receptors and enzymes were employed (Applied Biosystems, Life Technologies and Invitrogen, Stockholm, Sweden). Each sample was run in triplicate, and the transcript quantity was normalized with the $2^{\widehat{}}$(minCT-Ct) formula and the geometric mean of reference genes to the mRNA level of *cyclophilin A* (*PPIA*), *polymerase 2* (*Pol2a*) and *hypoxanthine guanine phosphoribosyl transferase* (*HPRT*) (Applied Biosystems, Life Technologies, Stockholm, Sweden). Gene assays and code number for all receptors and enzymes are found in (Supplementary information) data S Table 1.

2.4. Immunohistochemical analysis of human islets of Langerhans

Pancreatic sections from 3 to 5 donors and sections of human small intestine (carcinoid tumor) were collected on slides and air dried overnight at 37 °C. Slides were deparaffinized as previously described [19]. Sections were incubated with primary antibodies, goat anti-5HT (20079; dilution 1:400) (MBL international cooperation, Woburn, MA, USA), goat anti-5HT receptor 1D (sc-5393; dilution 1:25) (Santa Cruz Biotechnology, Dallas, TX, USA), rabbit anti-5HT receptor 2A (LS-A1109; dilution 1:200) (MBL international cooperation, Woburn, MA, USA), rabbit anti-glucagon (7811; dilution 1:10000), (EuroDiagnostica, Malmö), guinea pig anti-proinsulin (9003; dilution 1:2500) (EuroDiagnostica, Malmö, Sweden), rabbit anti-somatostatin (20067, Immunostar, INC, Hudson) overnight at 4 °C in moisturizing chambers. Sections were rinsed in PBS with Triton X-100 for 2 × 10 min. Thereafter secondary antibodies were applied as previously described [20]. The specificity of immunostaining was tested using primary antisera pre-absorbed with homologous antigen (5-HT, H9523), (Sigma–Aldrich, Stockholm, Sweden) (5-HT1d receptor, sc-5393 P) (5-HT2a receptor, MC-P1109), (MBL international cooperation, Woburn, MA, USA) (100 μg of peptide/ml antiserum at working dilution).

Immunofluorescence was examined in an epi-fluorescence microscope (Olympus, BX60). By changing filters the location of the different secondary antibodies in double staining was determined. Images were captured with a digital camera (Nikon DS-2Mv).

2.5. Insulin, glucagon and 5-HT secretion in human islets of Langerhans

Human islets (30 non-diabetic donors and 4 T2D islet donors) were handpicked under a stereo-microscope. Islets were divided into groups of 5 size matched islets and pre-incubated for 30 min with secretion assay buffer containing 2.8 mM glucose, 114 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO4, 1.16 mM $MgSO_4$, 25.5 mM $NaHCO_3$, 20 mM Hepes, 2.5 mM $CaCl_2$, and 0.2% BSA (fatty acid free). Incubations with non-diabtic donors were performed using 2.8 mM and 16.7 mM glucose, including 5-HT (10 μM), the ago-