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Vesicular monoamine transporter, type 2 (vmat2) expression as it compares to insulin and pancreatic polypeptide in the head, body and tail of the human pancreas

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Abbreviations: BCM, β-cell mass; PP, pancreatic polypeptide; ^{11}C-DTBZ, ^{11}C-dihydrotetrabenazine; ^{18}F-FP-DTBZ, ^{18}F-fluoropropyl-dihydrotetrabenazine; PET, positron emission tomography; T1D, type 1 diabetes; VMAT2, vesicular monoamine transporter, type 2

The vesicular monoamine transporter, type 2 (VMAT2) is responsible for sequestering monoamine neurotransmitters into exocytic vesicles in neurons, enterochromaffin-like cells of the stomach and cells arising from the common myeloid progenitor. VMAT2 is also present in the pancreas and is expressed by insulin producing β cells, but not by glucagon or somatostatin expressing islet cells. Positron emission tomography (PET) targeting of VMAT2 is currently being evaluated as a non-invasive tool to measure β-cell mass (BCM) in living humans. In recent trials, PET measurements of VMAT2 in the pancreas overestimated BCM in type 1 diabetes (T1D) patients predicted to have little to no BCM by metabolic measures. Recently, tissue immunohistochemistry studies suggested that VMAT2 staining may also co-localize with pancreatic polypeptide (PP) staining cells in pancreas tissue, but these studies were not quantitative. In this report, we evaluated VMAT2 specificity for β cells in sub-regions of the human pancreas using antibodies targeting VMAT2, insulin and PP by double-label immunofluorescence. Immunostaining for VMAT2 and insulin demonstrated 89 ± 8% overlap in the body and tail of the pancreas. However, 44 ± 12% and 53 ± 15% of VMAT2 cells co-stained with PP- and insulin-staining cells, respectively in the pancreatic head. Significant co-staining for VMAT2 and PP cells in the head of the pancreas may partly explain the apparent overestimation of BCM in T1D by PET. Specific targeting of the pancreatic body and tail using VMAT2 PET scanning may reflect BCM more accurately.

Introduction

Diabetes mellitus occurs when pancreatic β-cell mass (BCM) is reduced or destroyed. In type 1 diabetes (T1D), there is a near total loss of BCM through autoimmune processes.[1] Type 2 diabetes (T2D) occurs after increasing insulin requirements are unsustained within the pancreas. In autopsy studies, T2D pancreata show an increased BCM compared with lean non-diabetic controls, but reduced compared with age and weight-matched controls.[2] Although there is a strong understanding of BCM dynamics in diabetes, tools that measure real-time progression and losses in humans may allow for enhanced treatment and prevention.[3,4] Our group and others are working to develop a reliable and accurate non-invasive in vivo measure of BCM via positron emission tomography (PET) scanning.[5]

The vesicular monoamine transporter type 2 (VMAT2) is a proposed surrogate measure of BCM in living humans.[6] VMAT2 is a transmembrane protein that translocates monoamines, such as catecholamines, from the cytoplasm into exocytic vesicles and is found in cells of the nervous, hematopoietic and neuroendocrine systems.[7] It has also been shown to co-localize with islet cells of the endocrine pancreas.[7,8] Initial studies demonstrated exclusive VMAT2 co-localization within β cells[7] and suggested a role in regulating insulin secretion.[9]

Radio-labeled ^{11}C- and ^{18}F-fluoropropyl-dihydrotetrabenazine (^{11}C-DTBZ and ^{18}F-FP-DTBZ) are ligands used in PET that specifically bind VMAT2 potentially targeting β cells. In rodent studies, ^{11}C-DTBZ PET measurements mirrored the loss of BCM.[10] In human subjects with long-standing T1D and very low c-peptide levels, ^{11}C-DTBZ uptake was approximately 28–40% the level of controls,[11] which was higher than expected for subjects

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Table 1. Insulin, PP and V MAT2 percent areas as a function of total pancreas area

Hormone	Insulin	PP	VMAT2
Region	Area density (%*)	Area density (%)	Area density (%)
Average hormone staining area over whole pancreas	1.51 ± 0.15£	0.81 ± 0.19	1.44 ± 0.35££
Average hormone staining area relative to section area by pancreas sub region			
Head	1.07 ± 0.23	1.58 ± 0.40¥,¥¥	1.39 ± 0.21
Body	1.59 ± 0.15**	0.58 ± 0.21	1.45 ± 0.15
Tail	1.69 ± 0.18***	0.41 ± 0.19	1.47 ± 0.35

*± Standard error of the mean. **Insulin staining in body significantly different than PP ($p = 0.04$). ***Insulin staining in tail significantly different than PP ($p < 0.0003$). ¥PP staining in head significantly higher body ($p < 0.03$). ¥¥PP staining significantly higher in head than tail ($p < 0.008$). £Insulin staining significantly higher than PP in total pancreas ($p < 0.003$). ££VMAT2 staining significantly higher than PP in total pancreas ($p = 0.013$). Regional and total pancreas section areas of insulin, PP and VMAT2 staining percentages are also noted. Measurements were taken on images at 20 × magnification. At least 50 islets (with minimum of four β cells per islet in the plane of the section) were selected at random. Areas were calculated using QCapture software and Microsoft Excel.

with presumed negligible BCM. A second recently published report found similar results using ^{18}F-FP-DTBZ.[12] The results of these studies have prompted questions regarding radioligand specificity and/or binding to cells other than β cells.[4] Researchers have suggested that higher-than-expected levels of radioligand in the pancreas may be related to non-specific uptake or off target (non VMAT2) binding.[13-15]

This high DTBZ PET uptake may also be related to VMAT2 co-localization with pancreatic cells other than β cells. In 2008, Saisho et al. reported that VMAT2 also co-localizes with pancreatic polypeptide-staining (PP) cells.[16] In their study, they found that 39 ± 7% of VMAT2 cells also expressed PP. Therefore, PP may contribute to DTBZ uptake on PET. Yet Saisho et al. did not quantify PP and VMAT2 co-localization throughout the pancreas, which is important when considering its distribution is mainly found in the region of the pancreatic head.[17] Therefore, we aimed to address the relative co-localization of insulin- and PP-staining cells to VMAT2 in specific regions of the pancreas (i.e., head, body and tail). The data collected might provide further data toward explaining higher than expected DTBZ pancreatic PET uptake in humans with T1D and/or offer methods to more specifically target β cells using PET.

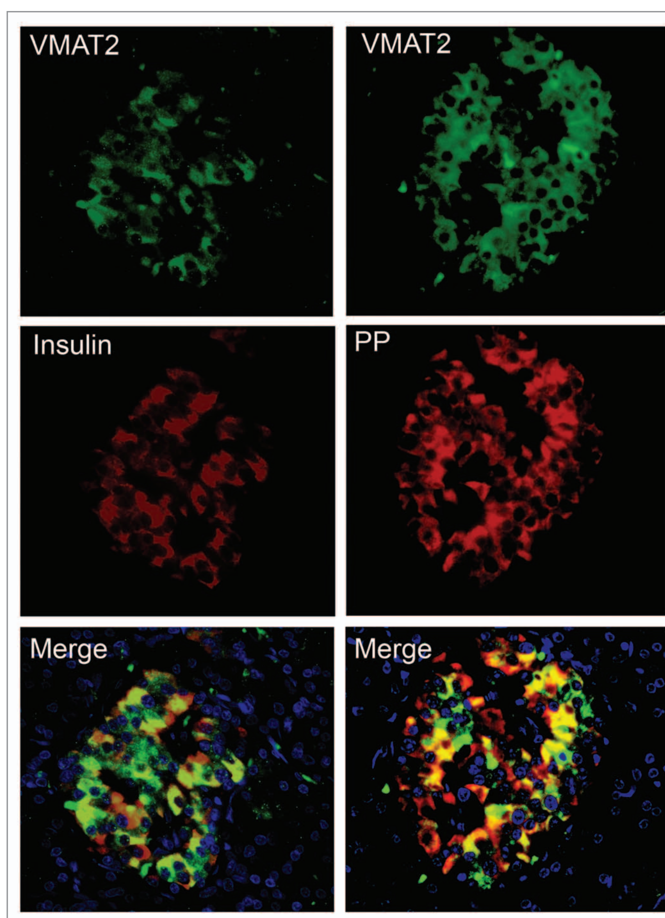

Figure 1. Immunofluorescent staining of the pancreatic head region of a non-diabetic subject. The left column represents VMAT2 (green), insulin (red), DAPI (blue) and merged (yellow) staining within an islet. The right column represents VMAT (green), PP (red), DAPI (blue) and merged (yellow) staining within a different islet.

Results

Pancreatic insulin, VMAT2 and PP expression. Mean percent staining areas for insulin, PP and VMAT2 for whole pancreas and regional distributions as well as further sub-classifications according to co-localization with islets or specific hormonal staining are shown in **Table 1** ($n = 8$). The mean insulin staining area, expressed as a percent of the whole pancreas was 1.51 ± 0.15%. It was no different than VMAT2 staining area (1.44 ± 0.35%, $p = 0.39$). Pancreatic polypeptide was significantly lower than insulin (0.81 ± 0.19%, $p < 0.003$) and VMAT2 ($p = 0.013$) staining.

Regional distribution analyses (i.e., head, body and tail) showed insulin and VMAT2 staining areas were similar in the body and

tail of the pancreas (**Fig. 1**; **Table 2**). In comparison, insulin expression in the pancreatic head was not significantly reduced as compared with VMAT2 (1.07 ± 0.23% vs. 1.39 ± 0.21%, p = 0.17). Pancreatic polypeptide expression was significantly higher in the head (1.58 ± 0.40%) than body (0.58 ± 0.21%, p < 0.03) or tail (0.41 ± 0.19%, p < 0.008). Pancreatic polypeptide was no different than insulin in the head of the pancreas (1.58 ± 0.40% vs. 1.07 ± 0.23%, p = 0.15); it was significantly lower than insulin content in the tail (0.41 ± 0.19% vs. 1.69 ± 0.18%, p < 0.0003).

VMAT2, insulin and pancreatic polypeptide co-localization by immunofluorescent imaging. As previously reported, VMAT2 primarily co-localizes with insulin-staining cells of the pancreas.[7] Approximately 94.7 ± 0.4% of insulin-staining islet cells co-localize with VMAT2. Co-localization of insulin with VMAT2 is weaker outside islets (37 ± 3%). Consistent with previous reports, VMAT2 co-localized with PP[16] and other cells,[18] but less than insulin. Approximately 36 ± 15% of PP-staining cells within islets co-localize with VMAT2; it is reduced outside islets (17 ± 6%). **Figures 1 and 2** illustrate typical co-localization of VMAT2 with insulin and PP.

Insulin immunoreactivity co-localizes significantly with VMAT2 in the pancreas. When reviewed regionally, VMAT2 primarily co-localizes with insulin in the combined body and tail (89 ± 8%). In the head, insulin and PP co-localize at similar levels with VMAT2 (insulin 53 ± 15%, PP 44 ± 12%).

VMAT2, insulin and pancreatic polypeptide immunofluorescence in long-standing T1D. Similar to previously reported data by Saisho et al.,[16] we found that VMAT2 expression was minimal in the tail region of the pancreas in long-standing T1D. Expression in the head of the pancreas, where PP is more abundant has not yet been reported. In this study, VMAT2 was present in the head of the pancreas despite no insulin immunoreactivity. VMAT2 co-localized with PP in the head of pancreas, similar to controls without diabetes (see **Fig. 3**).

Table 2. VMAT2 co-localization with insulin or PP

VMAT2 staining in pancreas sub-region	
Pancreatic head	
Associated with Islets	
Co-localized with Insulin	53 ± 15%
Co-localized with PP	44 ± 12%
Not associated with Islets	5 ± 1%
Pancreatic body	
Associated with islets	
Co-localized with Insulin	81 ± 12%
Co-localized with PP	12 ± 4%
Not associated with Islets	7 ± 1%
Pancreatic tail	
Associated with islets	
Co-localized with Insulin	96 ± 4%
Co-localized with PP	2 ± 1%
Not associated with Islets	2 ± 1%
Pancreatic body and tail combined	
Associated with islets	
Co-localized with Insulin	89 ± 8%
Co-localized with PP	7 ± 1%
Not associated with Islets	5 ± 1%

Co-localization is listed inside or outside islets. VMAT2 stains primarily within the islet. VMAT2 primarily co-localizes with insulin in the body and tail (81% and 96%). VMAT2 co-localizes nearly equally with insulin and PP in the head of the pancreas.

Discussion

Non-invasive measures of BCM in living humans may provide important pathophysiologic clues related to diabetes mellitus. The vesicular monoamine transporter, type 2 is a proposed surrogate marker of BCM. ^{11}C-DTBZ and ^{18}F-FP-DTBZ PET studies targeting VMAT2 have shown unexpectedly elevated pancreatic binding of tracer in humans with long-standing T1D.[11,12] The unexpectedly high binding of tracer may be related to non-specific binding, or binding to other cell types. Saisho et al. was the first to show that VMAT2 was not exclusively expressed by β cells but also co-localized with some PP-staining cells.[16] Such PP expressing cells persist in the pancreas even in T1D of long duration[17,19] possibly offering an explanation for the elevated tracer binding observed in DTBZ PET studies of T1D patients. The relative percent of immunostaining contribution of insulin and PP to VMAT2 was not fully explored, nor was regional distribution evaluated. Thus, we evaluated VMAT2, insulin and PP immunostaining in the whole and regional pancreas to assess the potential efficacy of VMAT2 imaging as a surrogate marker of BCM.

Our data are consistent with Saisho et al.;[16] VMAT2 co-localizes with both insulin and PP-staining cells. Furthermore, we found that approximately 81% and 96% (pancreatic body and tail, respectively) of VMAT2-stained cells co-localize with β cells. In the pancreatic head, VMAT2 co-localization is split evenly between insulin and PP (53% and 44%, respectively). Additionally, VMAT2 rarely co-localizes with other cell types including glucagon and somatostatin staining cells. These data also suggest nerves innervating islets and pancreas account for minimal, if any VMAT2.

Our data also confirm previously published studies describing insulin and PP distribution in the human pancreas.[17,20,21] Rahier et al. described endocrine cell distribution in the human pancreas.[20] PP cells were most abundant in the posterior head, up to 70% of all endocrine cells in the region. PP cells were rarely seen in the remainder of the pancreas, including the anterior head. Beta cells comprised as little as 20–25% of islet cells in the posterior head, but the great majority in the remainder of the pancreas (approximately 70%). We also found the highest abundance of PP in the head of the pancreas. PP-staining cells were more common in the body and tail sections than reported by Rahier et al.[20] In our study, β cells outnumbered PP cells 3:1 in the tail of the pancreas.

This study confirms that VMAT2 primarily co-localizes with insulin staining cells of the pancreas. More so, VMAT2 primarily co-localizes with β cells in the body and tail. β cell co-localization is much lower in the pancreatic head. Based on these

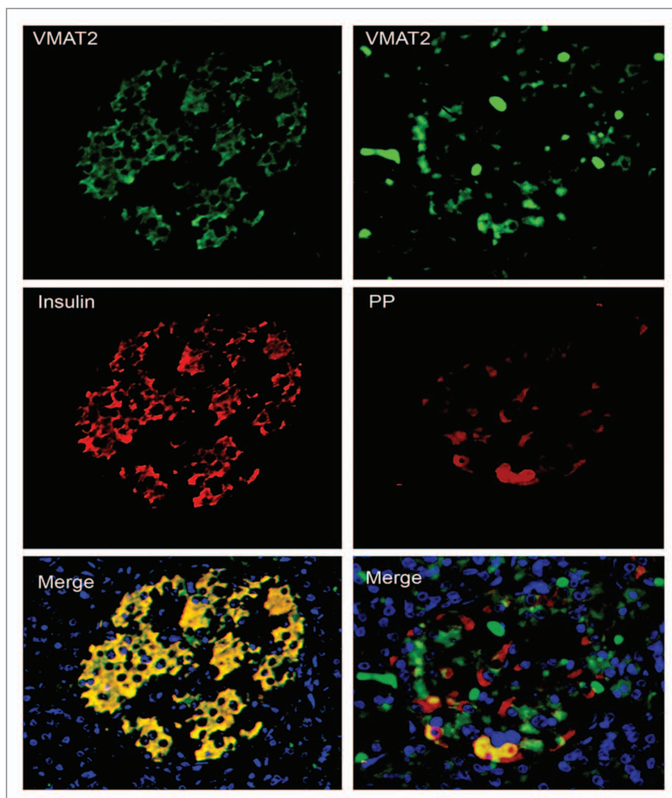

Figure 2. Immunofluorescent staining of the pancreatic tail region of a non-diabetic subject. The left column represents VMAT2 (green), insulin (red), DAPI (blue) and merged (yellow) staining within an islet. The right column represents VMAT (green), PP (red), DAPI (blue) and merged (yellow) staining within a different islet.

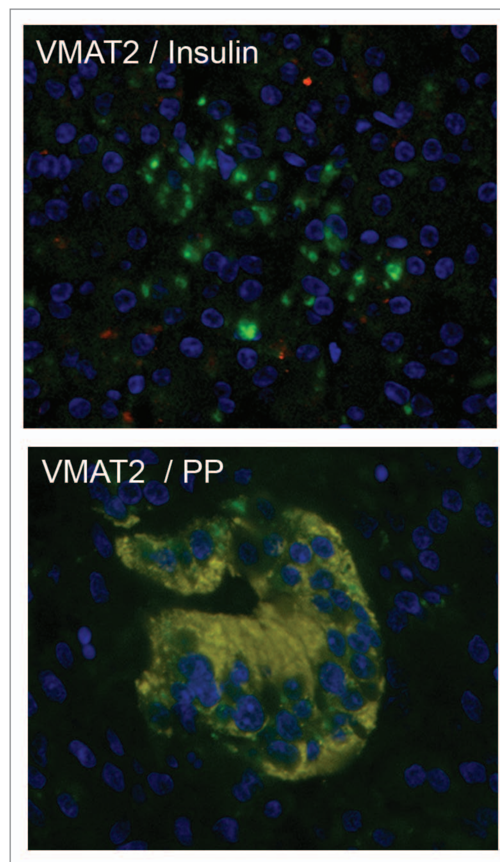

Figure 3. Immunofluorescent staining of the pancreatic head region of long-standing T1D. The top image shows VMAT2 (green) and DAPI (blue). There is rare insulin staining (red) or merge (yellow) as expected in T1D. The bottom image represents VMAT (green), PP (red), DAPI (blue) and merged (yellow) staining.

results, in vivo DTBZ PET pancreatic imaging may more specifically target true β-cell mass (in the body and tail regions) and provide more consistent data regarding actual BCM if VMAT2 quantitation in the pancreas excludes the head of the pancreas where VMAT2-positive PP cells are abundant. Future studies using ^{18}F-FP-DTBZ to measure in vivo β-cell mass may be guided by these results.

Materials and Methods

Subjects and pancreatic tissue. Whole pancreata received fixed in 10% formalin solution were procured from the National Disease Research Interchange (NDRI) or nPOD. IRB approval was obtained from Columbia University Medical Center and the study was performed in accordance with the Helsinki Declaration of 1975. Pancreata from subjects ranging in age from 58 to 70 y were sent from NDRI. Pancreas from five deceased male and three female subjects were used in this study. Other pancreata sent from NDRI were evaluated but excluded if the subject had a history of diabetes, autolysis was present, or if regions of interest (i.e., head, body or tail of pancreas) were not complete or could not be identified.

Tissue preparation, immunohistochemistry and analysis. Pancreatic tissue cut from each region of interest (i.e., head, body and tail). Tissue was embedded in paraffin and cut into five micrometer sections and prepared on slides by the CU Pathology Department. Next, sections were stained with primary antibodies against insulin (AB63820 Abcam, 1:200 or A0564 DAKO, 1:500), PP (NB-100-1793, Novus Biologicals 1:100 or A0619 DAKO, 1:2000) and VMAT2 (AB1767 Millipore 1:100 or 70808 Abcam 1:100). Secondary antibodies conjugated to horseradish peroxidase and diaminobenzidine oxidation were used to visualize insulin-, VMAT2- and PP-stained sections by light microscopy as per manufacturer's recommendations (Vector Laboratories). At least five tissue sections (> 5 cm^2 total) were evaluated for each subject, in each pancreatic region of interest and for each antibody pair used. We used Image Pro-Plus (7.0) software on a Nikon Eclipse E400 microscope and Prior automated stage. Methods to obtain staining areas are previously described.[16]

Immunofluorescence and co-localization. Co-localization was determined by double-label direct immunofluorescent staining using anti-VMAT2 antibody conjugated to APEX™ Alexa Fluor 488 (Life Technologies) and fluorescein conjugated anti-PP or -insulin antibodies. Methods and antibody specificity are previously described.[22] Background staining for insulin,

VMAT2, and PP was determined for each antibody (directly-labeled antibodies) or antibody pair. Antibodies were incubated on paraffin-embedded slides overnight at 4°C with the exception of insulin (2 h at room temperature). Slides were stained on a DAPI background and analyzed within one day using a Nikon Eclipse 80i microscope and QCapture 51 to image VMAT2, insulin and PP immunofluorescent location and co-localization. A limited amount of glucagon or somatostatin co-staining with VMAT2 was performed and confirmed not to co-localize.

Statistical analysis. Data are presented as mean ± SEM. Student's t-testing was used to compare results. A p value of < 0.05 was considered statistically significant.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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References

1. Meier JJ, Bhushan A, Butler AE, Rizza RA, Butler PC. Sustained beta cell apoptosis in patients with long-standing type 1 diabetes: indirect evidence for islet regeneration? Diabetologia 2005; 48:2221-8; PMID:16205882; http://dx.doi.org/10.1007/s00125-005-1949-2
2. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 2003; 52:102-10; PMID:12502499; http://dx.doi.org/10.2337/diabetes.52.1.102
3. Paty BW, Bonner-Weir S, Laughlin MR, McEwan AJ, Shapiro AM. Toward development of imaging modalities for islets after transplantation: insights from the National Institutes of Health Workshop on Beta Cell Imaging. Transplantation 2004; 77:1133-7; PMID:15114073; http://dx.doi.org/10.1097/01.TP.0000113231.90613.0E
4. Andralojc K, Srinivas M, Brom M, Joosten L, de Vries IJ, Eizirik DL, et al. Obstacles on the way to the clinical visualisation of beta cells: looking for the Aeneas of molecular imaging to navigate between Scylla and Charybdis. Diabetologia 2012; 55:1247-57; PMID:22358499; http://dx.doi.org/10.1007/s00125-012-2491-7
5. Freeby M, Goland R, Ichise M, Maffei A, Leibel R, Harris P. VMAT2 quantitation by PET as a biomarker for beta-cell mass in health and disease. Diabetes Obes Metab 2008; 10(Suppl 4):98-108; PMID:18834437; http://dx.doi.org/10.1111/j.1463-1326.2008.00943.x
6. Ichise M, Harris PE. Beta-cell Imaging: Opportunities and Limitations. J Nucl Med 2011; 52:493-5; PMID:21321277; http://dx.doi.org/10.2967/jnumed.110.086249
7. Anlauf M, Eissele R, Schäfer MK, Eiden LE, Arnold R, Pauser U, et al. Expression of the two isoforms of the vesicular monoamine transporter (VMAT1 and VMAT2) in the endocrine pancreas and pancreatic endocrine tumors. J Histochem Cytochem 2003; 51:1027-40; PMID:12871984; http://dx.doi.org/10.1177/002215540305100806
8. Maffei A, Liu Z, Witkowski P, Moschella F, Del Pozzo G, Liu E, et al. Identification of tissue-restricted transcripts in human islets. Endocrinology 2004; 145:4513-21; PMID:15231694; http://dx.doi.org/10.1210/en.2004-0691
9. Raffo A, Hancock K, Polito T, Xie Y, Andan G, Witkowski P, et al. Role of vesicular monoamine transporter type 2 in rodent insulin secretion and glucose metabolism revealed by its specific antagonist tetrabenazine. J Endocrinol 2008; 198:41-9; PMID:18577569; http://dx.doi.org/10.1677/JOE-07-0632
10. Souza F, Simpson N, Raffo A, Saxena C, Maffei A, Hardy M, et al. Longitudinal noninvasive PET-based beta cell mass estimates in a spontaneous diabetes rat model. J Clin Invest 2006; 116:1506-13; PMID:16710474; http://dx.doi.org/10.1172/JCI27645
11. Goland R, Freeby M, Parsey R, Saisho Y, Kumar D, Simpson N, et al. 11C-dihydrotetrabenazine PET of the pancreas in subjects with long-standing type 1 diabetes and in healthy controls. J Nucl Med 2009; 50:382-9; PMID:19223416; http://dx.doi.org/10.2967/jnumed.108.054866
12. Normandin MD, Petersen KF, Ding YS, Lin SF, Naik S, Fowles K, et al. In vivo imaging of endogenous pancreatic β-cell mass in healthy and type 1 diabetic subjects using 18F-fluoropropyl-dihydrotetrabenazine and PET. J Nucl Med 2012; 53:908-16; PMID:22573821; http://dx.doi.org/10.2967/jnumed.111.100545
13. Fagerholm V, Mikkola KK, Ishizu T, Arponen E, Kauhanen S, Någren K, et al. Assessment of islet specificity of dihydrotetrabenazine radiotracer binding in rat pancreas and human pancreas. J Nucl Med 2010; 51:1439-46; PMID:20720057; http://dx.doi.org/10.2967/jnumed.109.074492
14. Eriksson O, Jahan M, Johnström P, Korsgren O, Sundin A, Halldin C, et al. In vivo and in vitro characterization of [18F]-FE-(+)-DTBZ as a tracer for beta-cell mass. Nucl Med Biol 2010; 37:357-63; PMID:20346875; http://dx.doi.org/10.1016/j.nucmedbio.2009.12.004
15. Tsao HH, Skovronsky DM, Lin KJ, Yen TC, Wey SP, Kung MP. Sigma receptor binding of tetrabenazine series tracers targeting VMAT2 in rat pancreas. Nucl Med Biol 2011; 38:1029-34; PMID:21982574; http://dx.doi.org/10.1016/j.nucmedbio.2011.03.006
16. Saisho Y, Harris PE, Butler AE, Galasso R, Gurlo T, Rizza RA, et al. Relationship between pancreatic vesicular monoamine transporter 2 (VMAT2) and insulin expression in human pancreas. J Mol Histol 2008; 39:543-51; PMID:18791800; http://dx.doi.org/10.1007/s10735-008-9195-9
17. Malaisse-Lagae F, Stefan Y, Cox J, Perrelet A, Orci L. Identification of a lobe in the adult human pancreas rich in pancreatic polypeptide. Diabetologia 1979; 17:361-5; PMID:395002; http://dx.doi.org/10.1007/BF01236270
18. Rodriguez-Diaz R, Abdulreda MH, Formoso AL, Gans I, Ricordi C, Berggren PO, et al. Innervation patterns of autonomic axons in the human endocrine pancreas. Cell Metab 2011; 14:45-54; PMID:21723503; http://dx.doi.org/10.1016/j.cmet.2011.05.008
19. Gepts W, De Mey J, Marichal-Pipeleers M. Hyperplasia of "pancreatic polypeptide"-cells in the pancreas of juvenile diabetics. Diabetologia 1977; 13:27-34; PMID:320079; http://dx.doi.org/10.1007/BF00996324
20. Rahier J, Wallon J, Henquin JC. Cell populations in the endocrine pancreas of human neonates and infants. Diabetologia 1981; 20:540-6; PMID:6116638; http://dx.doi.org/10.1007/BF00252762
21. Gersell DJ, Gingerich RL, Greider MH. Regional distribution and concentration of pancreatic polypeptide in the human and canine pancreas. Diabetes 1979; 28:11-5; PMID:759245; http://dx.doi.org/10.2337/diabetes.28.1.11
22. Meier JJ, Butler AE, Saisho Y, Monchamp T, Galasso R, Bhushan A, et al. Beta-cell replication is the primary mechanism subserving the postnatal expansion of beta-cell mass in humans. Diabetes 2008; 57:1584-94; PMID:18334605; http://dx.doi.org/10.2337/db07-1369