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Neurofunctional Imaging of β -Cell Dynamics

Paul E. Harris and Rudolph L. Leibel

The Naomi Berrie Diabetes Center and Columbia University College of Physicians and Surgeons.

Abstract

Here we outline how islet cells use autocrine and paracrine “circuits” of classical neurotransmitters and their corresponding receptors and transporters to communicate with vicinal β -cells to regulate glucose stimulated insulin secretion. Many of these same circuits operate in the CNS and can be visualized by molecular imaging. We discuss how these techniques might be applied to measuring the dynamics of β -cell function in real time.

Keywords

β -cells; imaging; GSIS; PET

Introduction

An important issue in the pathogenesis of diabetes, in all of its molecular incarnations, is the relative contributions of reductions of β -cell mass and impairments in function of individual islet cells. For example, while destruction of β -cells is the primary pathogenetic process in T1D, hypofunction of the remaining β -cells - due to the effects of ambient cytokines and metabolites – clearly contributes. In T2D, metabolic stress on β - cells is the major mechanism for relative hypoinsulinemia, but the mass of β -cells may ultimately decrease as a result of this process, exacerbating the defining systemic pathology [1]. These are not simply fine points. Ultimately, the management and prevention of the disease hinges on these distinctions. To make such distinctions, what is needed are methods that can quantify β -cell mass *in vivo*, while, ideally also characterizing functional competence of the β -cell.

Our group has taken advantage of the fact that many of the β -cell-restricted “neuroreceptors” and transporters are quantifiable by non-invasive PET imaging. The β -cell-restricted patterns of expression of these molecules allow us to make *in vivo* measurements of β -cell mass and function [2-4] within the pancreas. This overview is aimed at summarizing studies that have lead to a broader understanding of the possible roles of the “classical” neurotransmitters and receptors have in regulating GSIS and address how molecular imaging might be applied to capture information regarding BCM, function and dysfunction *in vivo*.

Address Correspondence to: Paul E. Harris, Ph.D. Division of Endocrinology, Department of Medicine Columbia University Medical College 650 West 168th Street, BB 2006 New York, New York 10032 Tel: 212 305 7363 Fax: 212 305 7348 peh1@columbia.edu.

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Monoamine Neurotransmitter Regulation of Insulin Secretion

We and others have proposed a novel autocrine “circuit” of regulation of glucose-stimulated insulin secretion (GSIS) [5,6], mediated by monoamine neurotransmitters stored within islet vesicles. This system is distinct from that mediated by intrinsic parasympathetic-sympathetic innervation of islets (summarized by Ahrén [7]) which plays a central role in islet hormone secretion and glucose homeostasis (reviewed in [8]). In man, these autocrine (or paracrine) circuits may be particularly important because, relative to rodent islets, human islets appear more autonomous from CNS control, being sparsely innervated with few contacts to autonomic and cholinergic axons [9]. Moreover, in human islets, sympathetic axons are associated with the smooth muscle cells of blood vessels located around and deep within the islet rather than directly contacting β -cells. Effects on blood flow can, of course, have major impact on islet physiology [10].

The existence of an autocrine inhibitory circuit acting among the resident cells of islets is suggested by three lines of evidence: 1) islet cells contain vesicular stores of neurotransmitters (e.g. DA and 5-HT); 2) islet cells (i.e. β -cells) express receptors and transporters for neurotransmitters; 3) GSIS can be negatively regulated by DA and 5-HT and *in vitro* islets release specific monoamine neurotransmitters in proportion to ambient glucose concentrations [11-14]. Throughout the body, monoamine neurotransmitters including norepinephrine, dopamine, and serotonin are shuttled from the cytosol to secretory storage granules by vesicular monoamine transporters (VMATs) [15]. In the human pancreas, the expression of VMAT2 is largely confined to insulin-containing β -cell granules [16,17], and absent from islet cells staining for glucagon (α cells), somatostatin (δ cells); relatively minor VMAT2 expression occurs in PP cells and exocrine pancreas [17, 18]. Similarly, the pancreas also expresses dopamine type 2 receptors (D2R), and, like VMAT2, D2R expression within the pancreas is largely confined to β -cells [5]. On this basis, we propose a feed forward inhibitory circuit where DA, secreted along with insulin, down-regulates GSIS in islets via interaction with D2R (figure 1 and 2).

Intra-islet communication via other classical neurotransmitters has also been documented. Autocrine and paracrine signaling via γ -amino butyric acid (GABA) and the GABA alpha type receptors (GABARs) has been studied in rodent, and recently in human islets [19]. GABA was found to be released by cultured islets *in vitro*, in a glucose-dependent manner. Of note was GABA-enhanced GSIS in human islets, seemingly distinct from GABA's role in the CNS as an “inhibitory neurotransmitter.” Lastly, GABAR expression was found predominantly on cells staining for somatostatin (δ -cells), with some staining on subpopulations of alpha and β -cells. A second paracrine circuit between α -cells and β -cells has been described by Caicedo et al [20]. As mentioned above, α -cells do not express VMAT2. Instead, α -cell vesicles express vesicular acetylcholine transporter (VACHT), the molecule responsible for the vesicular storage of the acetylcholine. Acetylcholine (ACh) released by α -cells primes the β -cell insulin secretion via muscarinic M3 receptors to respond optimally to subsequent increases in ambient glucose concentration [21].

Both dopamine and serotonin are stored in β -cell vesicles and regulate GSIS

An appreciation of the presence of monoamine neurotransmitters within islets and the role they play in regulation of glucose secretion began in the 1960s. With the development of specific and sensitive fluorescent methods of detecting catecholamines and other biogenic amines, Falk and Hellman in 1963 demonstrated the presence of a fluorescent signal with granular distribution in “alpha” cells and “beta” cells within the islets of Langerhans of pancreata obtained from rats, mice, cats, dogs, horse and ducks. Presaging research that would take place 45 years later, these investigators also showed that the vesicular signal was blocked by reserpine (a VMAT2 inhibitor) [22]. Later these amines were shown to be 5-

hydroxytryptamine (5-HT or serotonin) and dopamine (DA) [23,24]. Using EM and autoradiography, 5-HT was found to be located in β -cell secretory granules [25,26]. In subsequent studies in rodents and humans, both *in vivo* and using islets *ex vivo* [27-30], it was shown that both 5-HT, L-DOPA (the direct metabolic precursor to DA) as well as DA could inhibit GSIS.

Since then it has become clear that not only do β -cells contain monoamine neurotransmitters, but that most if not all of the enzymes needed for *de novo* synthesis and catabolism of monoamines, including: tyrosine hydroxylase (TH), the enzyme catalyzing the rate limiting synthetic step whereby tyrosine is converted to L-DOPA [31]; L-DOPA decarboxylase (a.k.a. Aromatic L-amino acid decarboxylase (AADC) [5] producing DA; the catabolic enzymes acting on DA, monoamine oxidase (MAO-B) [32,33], AADC, and catechol-O-methyl transferase (COMPT) [34]. Recently, the gene expression pattern needed for encoding all of the products necessary for synthesizing, packaging, and secreting serotonin, including both isoforms of the serotonin synthetic enzyme tryptophan hydroxylase, have been found in islets [35].

Role of β -cell expression of D2R and 5-HTR

While evidence that *in vitro* GSIS could be modulated by exogenous DA and 5-HTR was provided years ago, experimental proof that β -cells express the corresponding receptors for DA and 5-HTR is recent and incomplete. Both rat and human islets express mRNAs for the 5-HT type 2A/B receptor [36,37]; however demonstration of expression of 5-HTRs at the protein level is lacking or indirect [38]. In contrast, the evidence for β -cell expression of dopamine type 2 receptors is solid. Rubi et al [5] have shown that rat islets express the dopamine type 1-3 and 5 receptor message, and that human islets express the dopamine type 2 receptor protein in a pattern that overlaps with insulin staining, consistent with an intravesicular location. Interestingly, in D2R global knockout mice, *in vivo* GSIS is impaired, suggesting that while dopamine clearly inhibits insulin secretion, some low level of D2R occupancy may be required for normal insulin secretion [39]. Consistent with this formulation, *in vitro* studies of GSIS show that micromolar concentrations of DA (10^{-6} M) enhance insulin secretion while higher concentrations (10^{-5} to 10^{-3} M) inhibit secretion [30].

Role of monoamine transporters in GSIS

The key role of VMAT2 in transporting monoamines from the cytosol to the vesicular lumen for storage and the related medicinal chemistry of VMAT2 have been expertly reviewed elsewhere [40,41]. In the context of an autocrine circuit of regulation of GSIS, VMAT2 function constitutes a central element. In both rodent and human pancreata, VMAT2 is preferentially expressed by β -cells [6,16,17] relative to the exocrine pancreas. Tetrabenazine (TBZ) binds to VMAT2 with a K_i in the nanomolar range [42] and inhibits the transport of monoamines to vesicles from the cytosol [43]. In *in vitro* experiments with purified rodent islets exposed to 20 mM glucose, concurrent treatment of cultured islets with TBZ significantly enhanced insulin secretion. Similar results were reported *in vivo* using a rat model with intraperitoneal glucose tolerance testing. In these experiments, both exogenous L-DOPA and DA partially reversed the effects of systemic TBZ [6]. Measurements of total tissue dopamine revealed that TBZ-treated pancreata and brains were dopamine depleted as predicted by a model where dopamine not sequestered into vesicles is catabolized by MAO [44]. These data fit well with the dopamine autocrine inhibitory circuit proposed in figures 1 and 2.

In the CNS, DA released at the synapse is recycled into vesicles by the action of the dopamine transporter (DAT a.k.a. SLC6A3) [45]. There are few published data supporting the preferential expression of dopamine transporters (DAT) in β -cells or islets (relative to

the pancreas as a whole) beyond those found in the public gene expression data bases [46]. These data are, however, consistent with the preferential expression of DAT by islets relative to the total pancreas. Preliminary experiments with rodent islets suggest that DAT inhibitors have effects on GSIS similar to dopamine depleting agents (e.g. augment insulin secretion in response to glucose) (Simpson et al, *personal communication*). In DAT knockout mice there is a generalized depletion of dopamine levels in the CNS [47]. If inhibition of DAT in β -cells functions analogously to DAT knock out in the CNS and results in a depletion of dopamine levels in the islets, then enhanced insulin secretion is predicted by our model.

Role of neutral amino acid carriers

Given the importance of DA and 5-HT in regulating GSIS, and the observation that β -cells express the biosynthetic machinery to synthesize DA and 5-HT from metabolic precursors, it seems reasonable to posit that mechanisms related to import of tryptophan and tyrosine into β -cells could play an important role in the physiology of GSIS. The large neutral amino acid transporter (LAT1) (a.k.a. CD98) is a heterodimeric glycoprotein formed from SLC3A2 and SLC7A5 subunits that preferentially transports branched-chain and aromatic (i.e. tryptophan, tyrosine) amino acids [48]. In gene expression profiling using rat islets cultured in either high or low glucose, SLC3A2 is down regulated in response to high glucose [49], as might be predicted by the model given in figures 1 and 2. The collection of more information regarding β -cell expression and regulation of these transporters seems warranted.

Lastly, it is interesting to note that genes participating in pathways regulating the biosynthesis, catabolism and response to DA and 5-HT have been implicated in diabetes- and/or obesity-related phenotypes by genetic association studies [50].

Molecular Imaging of β -Cell Restricted Targets To Capture Dynamics of GSIS

Imaging β -cell mass via VMAT2

Because for VMAT2: 1) transcription appears to be stable *in vitro* under a variety conditions including a range of glucose and lipid concentrations (e.g. up to 20 mM glucose and 0.5 mM palmitic acid) ; 2) protein expression parallels insulin staining in pancreata of T2D and T1D patients [17]; and 3) density can be quantified by PET imaging [51], we proposed the use of VMAT2 as a biomarker of insulin producing β -cells mass (BCM) in the study of the pathogenesis and management of type 1 and type 2 diabetes. PET imaging of BCM via VMAT2 has been recently reviewed [3]. Recent data by two groups suggest that VMAT2 quantitation in the pancreas by PET (with ^{18}F -FP-DTBZ) can readily distinguish between the BCM phenotypes found in healthy controls and individuals with long standing T1D [52] (Freeby et al, *submitted*).

The pet ligand currently in use to test the feasibility of measuring human BCM (^{18}F -FP-(+)-DTBZ) binds to VMAT2 with high affinity [53]. Past clinical studies of PET imaging of BCM, however, used a different tracer (^{11}C)-(+)DTBZ) with lower affinity for the VMAT2 transporter, a consequence of which would be less precise BCM measurements [2]. Following the development of ^{18}F -FP-(+)-DTBZ, two papers questioned VMAT2 measurements as a stable dopamine (DA) neuron biomarker [54, 55]. One study demonstrated that in DOPA-responsive dystonia, a human neurological disorder resulting from the inability to synthesize DA, there was an apparent 20% increase in (^{11}C)-(+)DTBZ binding in pathologically relevant regions (e.g. striatum) of the CNS. Both investigative teams concluded that the increased [^{11}C]-(+)-DTBZ binding reflected the greater

accessibility of the radioligand to VMAT2 binding sites due to lack of competition from endogenous DA. Together these papers suggested that [^{11}C]-(+)-DTBZ binding to VMAT2 might be sensitive to changes in vesicular DA storage levels. In instances of dopamine depletion (i.e. chronic methamphetamine users and DOPA responsive dystonia), these reports found increased [^{11}C]-(+)-DTBZ binding (about 20%), but not increased VMAT2 density. As a follow up to these studies, we performed VMAT2 imaging in the rat brain with [^{11}C]-(+)-DTBZ. Prior to imaging, rodents were treated with alpha-methyl-p-tyrosine (AMPT, a tyrosine hydroxylase inhibitor) at doses that significantly (~75%) depleted brain tissue dopamine levels. Imaging revealed increased striatal binding of the [^{11}C]-(+)-DTBZ VMAT2 tracer [56] in the AMPT-treated cohort relative to untreated controls. This reciprocal effect of ambient dopamine on [^{11}C]-(+)-DTBZ as a quantitative VMAT2 tracer may have partially confounded our previous PET measurements of BCM based on DTBZ binding to VMAT2 [2]. Differences in pancreatic dopamine content of the clinical cohorts studied could have influenced apparent BCM. Accordingly, we next examined the relationship between dopamine depletion and pancreatic VMAT2 - ^{18}F -FP-(+)-DTBZ binding *in vivo*. First we documented the *in vivo* effects of alpha methyl p- tyrosine (AMPT) administration in Lewis Rats. Adult rats were administered either a saline control injection or AMPT for several days and the tissues were analyzed for DA content. As expected, administration of AMPT reduced brain and pancreas DA content to 10-20% of the controls. To determine whether tissue DA content influenced the binding of ^{18}F -FP-(+)-DTBZ to VMAT2, we applied a serial pancreatic imaging strategy. Rodents were imaged at baseline with ^{18}F -FP-(+)-DTBZ and then again following the end of the course of AMPT. We found no statistically significant differences in the mean % change distribution volume ratio (DVR) in the dopamine depletion study (Table 1), suggesting that the dopamine status in the pancreas does not affect ^{18}F -FP-(+)-DTBZ tracer binding to VMAT2. In parallel studies no effects of dopamine depletion on tracer binding were seen in the CNS as well. A power analysis of the sample sizes used in this study indicated that there was at least an eighty percent chance to detect differences of 20% at 95% confidence interval. These results suggest that ^{18}F -FP-(+)-DTBZ binding to VMAT2 ($K_d = 0.5 \text{ nM}$) are not as sensitive to local dopamine levels as was observed for [^{11}C]-(+)-DTBZ binding to VMAT2 ($K_d = 1.7 \text{ nM}$) in the CNS because of the higher affinity of the former tracer for its target.

If one reconsiders the imaging properties of [^{11}C]-(+)-DTBZ, in terms of its sensitivity to the local concentration of monoamines, the possibility of imaging the dynamics of GSIS (e.g. changing concentrations of monoamines within the islet) becomes apparent. While this area is as yet unexplored in terms of measuring β -cell function, precedents in molecular imaging of the CNS support its feasibility. If β -cell VMAT2 expression is truly as invariant as evidence suggests, then the BCM measures provided by PET scans with ^{18}F -FP-(+)-DTBZ might serve as a convenient denominator of combined measures of both functional (e.g. using [^{11}C]-(+)-DTBZ as a displaceable tracer) and anatomical BCM (i.e. using the non-displaceable tracer ^{18}F -FP-(+)-DTBZ). In the field of CNS imaging, the majority of functional studies have centered on dopaminergic neurons and the measurement of changes in occupancy of the dopamine receptor.

Imaging D2R occupancy in islets as a measure of β -cell function

Dopaminergic neurotransmission has a critical role in movement coordination, and degeneration of the nigrostriatal dopamine system causes Parkinson's disease. Dopamine secretion and changes in D2R receptor occupancy in the CNS have been associated with a reward system that may impact drug abuse and food intake [57]. Imaging of neurotransmission and changes in D2R occupancy using PET and single photon emission computed tomography (SPECT) has been reviewed [58] and is an accepted technique in the study of CNS disorders such as schizophrenia. For example, healthy control subjects were

imaged at baseline with the D2R SPECT tracer [^{123}I] IBZM, after the first imaging session, but while the subject was still in the camera, dextroamphetamine sulfate, a compound known to increase synaptic dopamine concentration, was administered and the subject reimaged. A third scan was performed after a three day course of oral AMPT. The model that was applied to the analysis of the data predicts that challenges (e.g. GSIS) that increased local (i.e. synaptic or inter β -cell) DA concentration will increase occupancy of D2 receptors, reducing availability of D2 receptors for binding of the radiotracer. Conversely, conditions that decrease DA synaptic concentration (e.g. treatment with tetrabenzine) will reduce D2 receptor occupancy by DA and increase D2 receptor availability for radiotracer binding [58]. Comparison of the apparent binding density of the tracer revealed that relative to baseline, amphetamine decreased in the amount of tracer bound, while dopamine depletion by AMPT was associated with an increase in the amount of bound tracer [59].

Within the human pancreas, D2R expression is restricted to β -cells and is at least 50 fold higher in islets relative to the surrounding exocrine tissue (Maffei, *personal communication*). As posited in our model of the dopaminergic regulation of insulin homeostasis, GSIS may be accompanied by changes in numbers of functional D2R as well as by changes in D2R occupancy by dopamine. The feasibility of imaging of dopamine receptors as a biomarker of β -cell mass has already been demonstrated in rodents by Garcia et al [60], but our interest in this imaging approach is to capture in real time possible changes in pancreatic D2R occupancy that may accompany GSIS. If feasible, such measurements would yield information regarding β -cell function relevant to a phenotype distinct from that measured by current metabolic assessment measures such as mixed meal or oral glucose tolerance testing.

We selected a high affinity D2R tracer, [^{11}C] isoremoxiopride (a.k.a. [^{11}C] FLB 457) ($K_d=0.02$ nM), whose binding to D2R has been reported to be less sensitive to local DA concentration compared to other D2R tracers such as [^{11}C] raclopride ($K_d=1.3$ nM) [61,62]. We also included in the study the VMAT2 tracer ^{18}F -FP-(+)-DTBZ as a control probe known to be selective for pancreatic β -cells. The quantitation of D2R in the pancreas was assessed using the following strategy: we dynamically imaged the abdomen of male baboons for 60 minutes. From reconstructed images of the PET data, we placed regions of interest on the pancreas and other abdominal organs to generate time-activity curves (TACs) from which tracer kinetics could be studied. From the TACs, using a reference region method, we estimated the tracer target density using the distribution volume ratio (DVR) as the outcome measure. Using this approach we performed serial imaging experiments and quantitated the apparent D2R density in the pancreas region of interest. Reconstructed PET images from scans performed with D2R tracer (i.e. [^{11}C] FLB 457) and VMAT2 tracer (^{18}F -FP-(+)-DTBZ) showed exact overlap of their respective pancreatic signals (figure 3) suggesting that that D2R and VMAT2 coexist within the pancreas consistent with immunohistochemistry of β -cells in pancreatic sections using D2R- and VMAT2-specific antibodies. Serial scans of the same baboon performed with D2R tracer alone, and then following prior administration of a D2R blocker (i.e. the D2R antagonist haloperidol), demonstrated that a significant portion (>50%) of the pancreas PET signal was specific (figure 4)

The next step in this series of experiments will be to image subjects serially before and after an intravenous glucose tolerance test (IVGTT). We hypothesize that the PET signal will change in the pancreas in a manner analogous to that observed in the brain following amphetamine challenge. For example, in the resting pancreas, PET scans with D2R tracers may reveal time-activity curves very similar to that illustrated in figure 3 (left top panel). Once β -cells are stimulated *in vivo* by IVGTT and reimaged, a muted time-activity curve similar to that illustrated in figure 3 (left bottom panel) may be produced. It is possible that the IVGTT will need to be performed during the imaging session; in this case, a change

in slope of the time activity curve might be expected during stimulation. The magnitude of the changes in the time-activity curves are expected to reflect a unique facet of β -cell function (i.e. changes in D2R receptor occupancy) related to, but distinct from, metabolic measures based on insulin production.

Imaging monoamine precursor metabolism with ^{18}F -DOPA

Since insulin resistance and hyperinsulinemia generally precede the onset of obesity-induced T2D, molecular imaging of these processes could hypothetically be used in diagnosis and the evaluation of existing and novel therapeutic interventions. Congenital hyperinsulinism of infancy (CHI) manifests as persistent hyperinsulinism and severe hypoglycemia (reviewed in [63]) and represent an example where molecular imaging has been successfully applied as a diagnostic measure. The metabolic changes that accompany CHI have been studied *in vivo* [64] and *in vitro* [65] and it is believed that the insulin hypersecretory phenotype is accompanied by hyperactivity of L-DOPA metabolism. As might be expected on the basis of clinical findings, *in vitro*, CHI β -cells secrete insulin tonically. *In vitro*, CHI β -cells release about 5x more insulin under basal glucose stimulation and under longer culture periods release 10-40 % more insulin than control tissue [65]. Severe CHI is frequently treated by near total pancreatectomy which often results in later development of diabetes [66]. CHI is most often associated with recessive, loss of function mutations in ABCC8 and KCNJ11. ABCC8 and KCNJ11 encode two subunits of the pancreatic ATP-sensitive potassium (KATP) channel [67]. There are two histopathological types of CHI, categorized as diffuse and focal. Diffuse CHI arises when mutations are present in both alleles of the KATP channel genes, resulting in insulin over-secretion from all β -cells in the pancreas. In focal CHI, there is specific loss of the maternal 11p15 region accompanied by a paternally inherited mutation of the KCNJ11 gene. The lost region of maternal 11p15 carries silenced genes regulating cell proliferation. The resultant paternal disomy at 11p15 and loss of maternal silenced genes leads to a growth advantage for insulin overproducing β -cells resulting in a focal lesion in the pancreas [68]. The critical difference is that focal CHI can often be treated by selective resection, rather than near total pancreatectomy.

PET scans with ^{18}F -DOPA are very useful in distinguishing focal from diffuse CHI [69,70]. The principle of the ^{18}F -DOPA PET scan is based on β -cell uptake of L-dihydroxyphenylalanine (L-DOPA) via the L system transporter previously mentioned for later conversion to DA.

A major question that needs to be addressed is whether ^{18}F -DOPA metabolism is sufficiently specific to visualize normal but hyperfunctioning β -cells. The entire normal pancreas shows uptake of ^{18}F -DOPA as demonstrated by the fact that the normal pancreas has a standardized uptake value (SUV) of about 4 [71]. SUV is a relative measure of tracer concentration in the region of interest (ROI) where the ROI uptake is compared to what the tracer concentration (activity/cc) would be had the total tracer dose been dispersed in a volume of water equivalent to the subject's weight. In focal CHI disease, the ratio of lesion SUV to normal tissue SUV is 1.5 [72]. Both observations suggest that β -cell use of monoamines can be followed using ^{18}F -DOPA imaging. Another concern is that while β -cells express AADC, the enzyme responsible for metabolizing L-DOPA to DA, adjacent alpha cells and exocrine tissue also express AADC [73]. Possibly a "pulse-chase" paradigm (e.g. administer ^{18}F -DOPA, then follow with L-DOPA) could be applied to selectively label β -cell accumulation of ^{18}F -DA. If feasible, changes in β -cell vesicular ^{18}F -DA contents could then be visualized during *in vivo* GSIS.

Other neurofunctional β -cell targets

Figure 2 shows three additional cell surface molecules that may provide means to image β -cell mass and/or function. However, the β -cell specificity and abundance of expression of these targets are not documented.

Brice et al [74] recently demonstrated that *in vitro* insulin secretion could be modulated by agonists of the mGluR5 receptor as well as by agonists to mGluR2/3 and mGluR4/6-8 families. At present, PET ligand radiochemists are working to develop novel ligands with which to image receptors of the glutamate and γ -aminobutyric acid (GABA) system, but few ligands are available. We selected the [3 H]-APB 688, specific for the mGluR5 receptor [75], to explore the feasibility of imaging human β -cell mass or function. Using human pancreas membranes and a radioreceptor assay, we found that the B_{max} (molar amount of ligand bound per mass of protein) was too low to support feasibility of PET imaging of this target. There are several lines of evidence that other receptors in the GABA system are abundant targets on β -cells and may be worthy of a second look once the appropriate tracer is found.

Two additional putative targets for imaging β -cell dynamics during GSIS are shown in figures 1 and 2: the dopamine “reuptake” transporter (DAT) and the serotonin receptor (5-HT_{2A/B}). Again evidence for the expression of DAT is based on *in vitro* GSIS experiments using purified islets in combination with specific inhibitors of DAT that show enhanced insulin secretion when DAT is inhibited. Most of the evidence for β -cell or islet expression of 5-HT_{2A/B} is based *in vitro* GSIS experiments using agonists and antagonists of serotonin receptors or gene profiling experiments of islets and β -cells [49]. If imaging of serotonin receptors in islet β -cells is practical, it is possible that measurements of serotonin receptor occupancy in the pancreas will provide further insight into the real-time function of β -cells. PET ligands for these receptors have already been developed for quantitation of targets in the CNS (see examples in figure 2) and await testing of applicability in β -cell imaging. The critical questions regarding feasibility of these approaches will be: 1) what is the specificity of expression on β -cells relative to exocrine tissue; 2) what is their abundance of expression; and 3) what is the magnitude of non-specific binding of the tracer relative to the specific and saturable tracer binding in the pancreas taken as a whole.

Summary

As emphasized in the introduction, the issue of the relative contributions of impairments of BCM and function to the pathogenesis, natural history and responses to interventions of the various clinical forms of diabetes remains unresolved. But answers to these questions are important to guiding strategies for prophylaxis and prevention. For example, ongoing debates regarding the mechanism(s) by which bariatric/ “metabolic” surgery ameliorates T2D might be enlightened by the ability to monitor *in vivo* the functional and structural consequences to the β -cell of such interventions [76,77]. While the absorbed radiation doses associated with these PET studies are not trivial (3-30x absorbed radiation of typical chest x ray), two or three of these studies could be performed per year without exceeding federal exposure guidelines for research volunteers and patients.

It is interesting to note that the brain is only a minor source of peripheral DA. A major source of peripheral DA is the gastrointestinal tract [78]. This fact, coupled with the finding that there are large post prandial elevations in serum DA in man [79], suggests that DA in its role of regulating β -cell insulin secretion represents an “anti incretin” hypothesized to account, in part, for the beneficial effects of bariatric surgery on T2D [80]. Here, surgical procedures that possibly lower circulating and pancreatic DA levels (e.g. sleeve gastrectomy with duodenal switch) might be predicted to enhance GSIS. Such changes in pancreatic DA levels following bariatric surgery, as well as changes in anatomical β -cell mass, could be

monitored both prior to and after bariatric surgery using the imaging techniques outlined above. Monitoring these novel phenotypes might provide insights to the mechanisms at work in metabolic surgery for reversal of T2D.

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Figure 1.

Schematic of a putative dopamine mediated autocrine negative feedback circuit in β -cells. Import of aromatic amino acids (Tyrosine [Try] and Tryptophan [Trp]) by neutral amino acid carriers (LAT1) from extracellular space occurs, followed by transformation of Tryp and Trp to L-DOPA and 5-hydroxy-tryptophan (5-OH-TRP) by the action of tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH). Both L-DOPA and 5-OH-TRP are transformed into dopamine (DA) and serotonin (5-HT) by the action of aromatic amino acid decarboxylase (AADC). Cytoplasmic DA or 5-HT are transported by VMAT2 into β -cell vesicles and later delivered to the intracellular space following glucose stimulated insulin secretion. Dopamine type 2 receptors are stored in vesicles, rendered inactive in the acid microenvironment. Extracellular dopamine (e.g. as elevated dopamine sulfate found in postprandial serum samples) can be shuttled inside the β -cell by the action of the dopamine transporter (DAT). DA and 5-HT not sequestered by the action of VMAT2 is exposed to the action of monoamine oxidase and destructively metabolized.

Figure 2.

Upon glucose stimulation, insulin (open hexagons), dopamine (black ovals) and dopamine type 2 receptors (stippled) are delivered to the extracellular space or plasma membrane. Dopamine diffuses to active cell surface D2R or D2R expressed on neighboring β -cell and binds to dopamine receptor (D2R) inhibiting further insulin secretion. The dopamine receptor type 2 PET ligands [^{11}C]-FLB457 or ^{18}F -Fallypride are shown in grey ovals. The VMAT2 antagonist dihydrotetrabenazine (DTBZ) prevents vesicular storage of dopamine by high affinity binding near the monoamine binding site. The VMAT2 PET ligand ^{18}F -FP-DTBZ (grey rectangle) is shown bound to VMAT2 (open circle with X). The tyrosine hydroxylase substrate and PET ligand ^{18}F DOPA is shown adjacent to its enzyme. PET ligands for the metabotropic glutamate receptor, ^{11}C -APB 688 (stippled oval bound to open reversed C); dopamine transporter, ^{11}C -PE2I; (grey rectangle bound to open doughnut) and serotonin receptor 5HT 2A , ^{11}C MDL 100907 (stippled triangle bound to open pie) are also shown.

Figure 3.

Papio Ursinus was imaged twice in one session for a study comparing the region of interest defined by a VMAT2 probe, ^{18}F -FP-(+)-DTBZ, and a D2R probe $[^{11}\text{C}]$ FLB 457. The $[^{11}\text{C}]$ study was performed first and activity from the probe allowed to decay for six half lives (^{11}C $T_{1/2}$ = 21 minutes) before injection of the second VMAT2 probe, ^{18}F -FP-(+)-DTBZ. Following reconstruction of the images the pancreatic region of interest was defined using the VMAT2 probe. The overlap of the D2R and VMAT2 signals was then examined (bottom row-Merged). All studies were approved by the Columbia Institutional Animal Care and Use Committees.

Figure 4.

[¹¹C] FLB 457 study in one baboon at the level of the pancreas at baseline and following D2R blockade with haloperidol. Images are summed axial frames from 6 to 30 min post-injection of a bolus of [¹¹C] FLB 457. Data are not normalized to injected activity. Time activity curves (blue squares—ROI on kidney, light blue dots – ROI on pancreas) were used to calculate a distribution volume ratio (DVR) using a reference region method. The dose of haloperidol (a D2R antagonist) administered (1 mg/kg) was expected to displace at least 30% of the specifically bound PET ligand. A kidney reference region was used to calculate the DVR. Haloperidol administration caused the apparent DVR to drop to 1.1 from 1.4.

Table 1

Average Pancreatic VMAT2 Density as DVR measured with 18F-FP-DTBZ

Rodent ID	Baseline	AMPT		% Change in DVR
14F	2.00	1.92		4.00
15E	1.97	2.11		-7.11
39C	1.75	1.78		-1.71
3A8	2.10	2.50		-19.05
330	1.50	1.27		15.33
			Mean % Change DVR	-1.71
			Variability	12.77