Published in final edited form as:

J Mod Opt. 2012 May 1; 59(9): . doi:10.1080/09500340.2012.674564.

Functional imaging of glucose-evoked rat islet activities using transient intrinsic optical signals

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Abstract

We demonstrate intrinsic optical signal (IOS) imaging of intact rat islet, which consists of many endocrine cells working together. A near-infrared digital microscope was employed for optical monitoring of islet activities evoked by glucose stimulation. Dynamic NIR images revealed transient IOS responses in the islet activated by low-dose (2.75mM) and high-dose (5.5mM) glucose stimuli. Comparative experiments and quantitative analysis indicated that both glucose metabolism and calcium/insulin dynamics might contribute to the observed IOS responses. Further investigation of the IOS imaging technology may provide a high resolution method for *ex vivo* functional examination of the islet, which is important for advanced study of diabetes associated islet dysfunctions and for improved quality control of donor islets for transplantation.

Keywords

intrinsic optical signal; functional imaging; rat islet; glucose stimulation

Diabetes has become a global epidemic. It is estimated that 200 million people are currently suffering from diabetes, and the number may increase to 366 million in 2030 [1,2]. It is well known that islet beta-cell dysfunction is at the center of all major forms of diabetes. Thus, better investigation of islet beta-cell function can provide insight into improved prevention and diagnosis of diabetes. Freshly isolated (intact and living) pancreatic islets have been extensively used as a simple preparation for the study of beta-cell function. Electrophysiological measurements, such as membrane potential recording, have been explored for functional study of the beta-cells [3,4], but simultaneous electrophysiological measurement of multiple beta-cells working together is difficult. In intact islets, beta-cells are coupled together as a network for effective glucose regulated insulin release [5,6]. This coupling enables them to achieve appropriate glucose dose response and release insulin in a synchronized oscillatory fashion, leading to the multi-scale temporal rhythms in blood insulin, which is important for insulin action, and is compromised in diabetes. Given the fact

that the calcium influxes are tightly correlated with insulin regulation, calcium fluorescence imaging has been explored for functional study of glucose-evoked betacell activities in intact islets [7]. However, phototoxicity of fluorescence dyes may render the evaluated islets no longer suitable for transplantation. Moreover, loading of fluorescence dyes into the center part of intact islets is difficult, although calcium imaging of islet periphery area is practical [8].

Without the requirement of exogenous chemicals, intrinsic optical signal (IOS) imaging promises a new method for functional investigation of the islet. Stimulus-evoked IOSs have been observed in excitable biological tissues, such as neural systems [9–12]. Transient IOSs have been detected from glucose activated insulin secreting INS-1 cells, which is a popular model for investigating diabetes associated beta-cell dysfunctions. In this paper, we demonstrate the feasibility of using IOS imaging for functional examination of intact islets which consists of many endocrine cells working together.

Freshly isolated rat islets were employed for this study. Rat islets were isolated by the Islet Resource Facility of the UAB Comprehensive Diabetes Center (IRF-UCDC) using established procedures [13]. Briefly, Lewis rats weighing 250–300 gm (Harland Laboratories; Indianapolis, IN) were digested (37°C) with 1 mg/ml Liberase TL (Roche; Indianapolis, IN). Following digestion, islets were purified by discontinuous gradient centrifugation (1.096, 1.081, and 1.037 g/ml; Mediatech, Inc.; Manassas, VA). Isolated islets were maintained in Miami 1A (10% FBS and 100 mg/dL glucose; Mediatech, Inc.) overnight and 37°C and 5% CO₂. Islets were attached overnight to Collagen I-coated (Rat tail; Invitrogen Corp., Carlsbad, CA) 6-well tissue culture plates. Following attachment, islets were incubated (1 hr) in KRBB containing 3.3mM glucose and subjected to glucose stimulation at described varying doses with sequential 1 hour intervals.

The experimental setup of IOS imaging was based on a near infrared (NIR) transmission microscope equipped with a digital camera. The same imaging system has been used to detect IOSs in individual INS-1 cells [14] and retinal neurons [15–17]. During the IOS recording, the islet was continuously illuminated by the NIR light source; two doses (2.75mM and 5.5mM) of glucose step change were used to stimulate the islet. For each experimental trial, both pre-stimulus and after-stimulus images were recorded. Dynamic differential image processing [18] was employed to calculate transient IOS changes correlated with islet activities. IOS images are presented in the unit of $\Delta I/I$, where ΔI is the stimulus-evoked dynamic optical change and I is the background light intensity (i.e. the value in the previous frame).

Figure 1(a) shows representative islet images before data processing. Figure 1(b) and 1(c) present typical IOS patterns elicited by 2.75mM and 5.5mM glucose-step stimuli, respectively. All raw images shown in this article were recorded with a speed of 10 frames per second. Each illustrated image in Figure 1 was averaged over 20 s interval (200 frames). In order to demonstrate the repeatability of the IOS response, two experimental trials were implemented for each dose stimulus. As shown in Figure 1(b) and 1(c), robust IOSs were detected in the islet. Transient IOSs showed time courses tightly correlated with the stimulation. Both positive (increasing) and negative (decreasing) IOS were observed in the glucose activated islet. Control IOS imaging of dead islets (which was treated by 70% ethanol) was also performed; no detectable IOS change was observed in the dead islet.

In order to compare time courses and signal magnitudes of islet activities elicited by low (2.75mM) and high (5.5mM) glucose stimuli, the number of image pixels with detectable optical change was quantitatively calculated over each IOS frame. Figure 2(a), which corresponds to the fourth frame in Figure 1w(c2), shows one representative image with

activated and silent pixels marked by white and black colors, respectively. Standard deviation (σ) of each pixel location was calculated over the 40-s pre-stimulus period. In each post-stimulus image, if the pixel value change, relative to pre-stimulus baseline, was larger than 3-sigma, it would be accounted as an activated pixel. The 3-sigma criterion corresponds to a confidence probability of 99.73%. Figure 2(b1)–2(b4) illustrate temporal changes of activated areas in Figure 1(b) and 1(c), respectively. As shown in Figure 2, low strength stimulation typically revealed a monophasic (T1) response; while a biphasic (T1 and T2) response was observed in the islet activated by high strength stimulation. With enhanced stimulation, the peak value (59% in Figure 2(b3); 57% in Figure 2(b4)) of activated area was increased, compared to low strength stimulation (7% in Figure 2(b1); 4% in Figure 2(b2)). However, time-delay (relative to the stimulus onset) of the peak magnitude was shortened with enhanced stimulation (Figure 2(c)). We speculate that the monophasic (Figure 2(b1) and 2(b2)) IOS response associated with low-dose stimulation might reflect glucose metabolism only. Without reaching a certain threshold, the glucose metabolism associated with low-dose stimulation might not be enough to activate islet beta-cells to produce calcium/insulin activities. The first-phase of the biphasic IOS response (Figures 2(b3) and 2(b4)) might reflect glucose metabolism; while the second-phase might reflect involvements of calcium/insulin dynamics of islet beta-cells.

As shown in Figure 1(c), detectable IOS was observed at the periphery area first. However, late phase of the IOS response was dominantly confined in the center area. In order to quantify spatiotemporal dynamics of the IOS response, the islet was separated into three zone areas (Figure 3(a)). Figure 3(b) shows IOS average of each zone area; Figure 3(c) illustrates IOS responses of individual pixels selected from the edge (zone 1), inner (zone 2), and center (zone 3) areas. As shown in Figure 3(b) and 3(c), edge area (red) of the islet was dominated by a positive-going response; while IOS response of inner (blue and black) areas consisted of both negative-going (early phase) and positive-going (late phase) changes. IOS averages of the zone 1 (red), zone 2 (blue), and zone 3 (black) reached magnitude peaks (arrowhead in Figure 3(b)) at 31 s, 45 s, and 60 s, respectively.

In summary, we have demonstrated IOS imaging of intact rat islet that consists of many endocrine cells working together. Dynamic IOS imaging disclosed dynamic optical changes, which might result from cellular (i.e. swelling or shrinking change of the cell body) [14] and/or sub-cellular (e.g. nuclear infoldings) [19] changes, associated with the glucose stimulation. Spatiotemporal characteristics of IOSs elicited by low-dose (2.75mM) and high-dose (5.5mM) glucose stimulation were quantitatively analyzed. We hypothesize that the monophasic (negative change) IOS response associated with low-dose stimulation reflected glucose metabolism; while biphasic IOS response evoked by high-dose stimulation involved both glucose metabolism and calcium/insulin dynamics. Spatial differences (Figure 3) of the IOS responses consistently support our hypothesis. As shown in Figures 1(c) and 3, the second-phase (positive change) IOS, which might relate to islet beta-cell activities, were dominantly distributed at inner area of the islet. It is well established that the rat islet consists of a beta-cell kernel at the center and several other endocrine cells in the periphery. We are currently pursuing concurrent IOS imaging and electrophysiological measurement to achieve further investigation of the relationship between glucose-evoked IOS response and islet beta-cell activation. Better understanding of the IOS sources and mechanisms may lead to a noninvasive and high resolution method for *ex vivo* functional examination of intact islets, which is important for: (1) clinical application, to provide a method for high throughput screening of donor islets prepared for transplantation; (2) preclinical research, to allow high spatiotemporal resolution investigation of diabetes associated beta-cell dysfunctions.

Acknowledgments

This research is supported in part by Dana Foundation, Eyesight Foundation of Alabama, National Science Foundation (CBET-1055889), National Institutes of Health (R21-RR025788, R21-EB012264, and P60-DK079626), and the Islet Resource Facility of the UAB Comprehensive Diabetes Center (IRF-UCDC).

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Figure 1.

(a) Raw image sequence of the rat islet before data processing. (b1, b2) IOS images of the islet activated by 2.75mM glucose stimulation. (c1, c2) IOS images of the islet activated by 5.5mM glucose stimulation. b1, b2, c1, and c2 were recorded from the same islet in sequence, with sequence gap interval of 1 h. (The color version of this figure is included in the online version of the journal.)

Figure 2.

(a) One representative image with activated and silent pixels marked by white and black colors, respectively. This image corresponds to the fourth frame in Figure 1(c2). (b1–b4) Dynamic ratio changes of activated areas in Figure 1(b1), 1(b2), 1(c1), and 1(c2), respectively. Only image pixels occupied by the islet (red circle in a) were used to calculate the ratio of the area with IOS response. (c) Peak times (T1 and T2 in b) of the IOS responses. R1–R4 correspond to b1–b4. (The color version of this figure is included in the online version of the journal.)

Figure 3.

(a, b) Enlarged pictures of the fourth and fourteen frames in Figure 1(c2). (c) Averaged IOS responses of the zone 1 (red), zone 2 (blue), and zone 3 (black). As shown in (a), the zone 1 includes islet area between red and blue curves; the zone 2 includes the islet area between blue and black curves; and the zone 3 includes the islet area within the black curve. (d) IOS responses of individual pixels selected from the zone 1 (traces 1–3), zone 2 (traces 4–6), and zone 3 (traces 7–9). (The color version of this figure is included in the online version of the journal.)