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Enhanced glucose-induced intracellular signaling promotes insulin hypersecretion: Pancreatic beta-cell functional adaptations in a model of genetic obesity and prediabetes

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ABSTRACT

Obesity is associated with insulin resistance and is known to be a risk factor for type-2 diabetes. In obese individuals, pancreatic beta-cells try to compensate for the increased insulin demand in order to maintain euglycemia. Most studies have reported that this adaptation is due to morphological changes. However, the involvement of beta-cell functional adaptations in this process needs to be clarified. For this purpose, we evaluated different key steps in the glucose-stimulated insulin secretion (GSIS) in intact islets from female *ob*/*ob* obese mice and lean controls. Obese mice showed increased body weight, insulin resistance, hyperinsulinemia, glucose intolerance and fed hyperglycemia. Islets from *ob*/*ob* mice exhibited increased glucose-induced mitochondrial activity, reflected by enhanced NAD(P)H production and mitochondrial membrane potential hyperpolarization. Perforated patch-clamp examination of beta-cells within intact islets revealed several alterations in the electrical activity such as increased firing frequency and higher sensitivity to low glucose concentrations. A higher intracellular Ca^{2+} mobilization in response to glucose was also found in *ob*/*ob* islets. Additionally, they displayed a change in the oscillatory pattern and Ca^{2+} signals at low glucose levels. Capacitance experiments in intact islets revealed increased exocytosis in individual *ob*/*ob* beta-cells. All these up-regulated processes led to increased GSIS. In contrast, we found a lack of beta-cell Ca^{2+} signal coupling, which could be a manifestation of early defects that lead to beta-cell malfunction in the progression to diabetes. These findings indicate that beta-cell functional adaptations are an important process in the compensatory response to obesity.

1. Introduction

Obese individuals are at increased risk for type 2 diabetes. Hyperinsulinemia along with low insulin sensitivity are frequently observed in obesity (Kahn et al., 2006). Although insulin resistance is present in most obese subjects, glucose intolerance and hyperglycemia are not necessarily found in these individuals. Indeed, compensatory adaptations in the pancreatic β-cells usually allow for higher pancreatic insulin release in order to maintain normoglycemic values (Kargar and Ktorza, 2008; Seino et al., 2011). However, when β-cell compensations fail to adapt to the increasing insulin requirements imposed by insulin resistance, glucose tolerance becomes deteriorated in obese individuals and, eventually, they can develop overt hyperglycemia and type-2 diabetes (Kahn et al., 2006). Several studies in animal models and humans have reported that the enhanced plasma insulin levels observed in insulin-resistant states, like in obesity, are likely related with increases in β-cell mass (Sachdeva and Stoffers, 2009; Saisho et al., 2013; Seino et al., 2011). In contrast, other studies in non-diabetic obese human subjects have shown that beta-cell mass was only moderately increased (Rahier et al., 2008) compared with controls or that there were no differences (Kou et al., 2013). However, less importance has been attributed to the involvement of the β-cell function in these compensatory responses (Hull et al., 2005). Consequently, changes in the β-cell stimulus-secretion coupling remain poorly characterized in obesity (Kargar and Ktorza, 2008; Seino et al., 2011).

Recently, our group reported that β-cells from high fat diet-induced obese mice display several functional adaptations. In this insulin-resistant state, β-cell compensations led to insulin hypersecretion, maintaining normal glycemia and glucose tolerance in

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obese mice (Gonzalez et al., 2013). However, in the progression from normoglycemia to overt diabetes, insufficient β-cell compensation to insulin resistance can result in a prediabetic condition characterized by impaired glucose tolerance and moderate hyperglycemia (Weir and Bonner-Weir, 2004). In order to analyze this prediabetic state, here we aimed to elucidate the functional changes in the β-cell glucose-stimulated insulin secretion (GSIS) using a model of genetic obesity. The leptin-deficient *ob*/*ob* mouse is characterized by marked obesity, insulin resistance, glucose intolerance, moderate hyperglycemia and elevated plasma insulin levels, but they do not develop overt type 2 diabetes (Coleman, 1978). Given that *ob*/*ob* mice have larger islets of Langerhans (Bleisch et al., 1952; Bock et al., 2003; Gepts et al., 1960) with a higher proportion of β-cells (Baetens et al., 1978; Gepts et al., 1960; Westman, 1968a, 1968b), they have been extensively used as a source of pancreatic islets. Although numerous investigations have used *ob*/*ob* islets for β-cell studies (Bergsten et al., 1994; Hellman, 1965, 1970), a detailed analysis of the potential functional adaptations in the different steps of the stimulus-secretion coupling is still lacking. Additionally, most data about the islet function come from isolated β-cells (Ahmed and Grapengiesser, 2001; Grapengiesser et al., 1988), an experimental model that can differ from the physiological scenario, as it has been reported using intact islets (Göpel et al., 1999, 2000, 2004). In the current study, we show in intact pancreatic *ob*/*ob* islets that improved performance in the majority of steps involved in GSIS would account for the high insulin secretion rate characteristic of hyperinsulinemic insulin-resistant conditions, like in obesity. Additionally, the present findings further support the wide plasticity and crucial adaptation of the β-cell secretory process in the compensatory responses of the endocrine pancreas.

2. Material and methods

2.1. Animals

All protocols were approved by our Animal Ethics Committee according to national regulations. Five-week-old female *ob*/*ob* mice (C57BL/6J background) were purchased from Harlan Laboratories (Barcelona, Spain) and lean females of matched age were used as controls. In electrophysiological experiments, *ob*/*ob* female animals (C57BL/6J background) were purchased from Janvier (Janvier Labs, Le Genest sur l'Isle, France). Animals were housed at 22 °C with a light cycle of 12 hours (8:00 am–8:00 pm) and had free access to water and standard chow. Experiments were performed when animals were 12 weeks old.

2.2. Plasma measurements and tolerance tests

Glucose and insulin plasma levels were measured by tail bleeding in fed state and during tolerance tests (Gonzalez et al., 2013). Plasma glucose was measured with a commercial glucometer (Accu-Chek) and plasma insulin by a commercial ELISA kit (Crystal Chemical). For the glucose tolerance test, animals were fasted for 12 hours before an intraperitoneal (i.p.) glucose injection (2 g/kg). Plasma glucose was measured at 0, 15, 30, 60, 90, 120 and 180 min and plasma insulin at 0 and 30 min after the glucose challenge. For the insulin tolerance test, fed animals were subjected to an i.p. insulin injection (1 UI/kg) and then, plasma glucose was measured at 0, 15, 30, 45 and 60 min. The HOMA-IR was also calculated as an indicator of insulin resistance: [fasted plasma glucose (mg/dL) * fasted plasma insulin (mU/L)]/405 (Solomon et al., 2014; Tripathy et al., 2010).

2.3. Islet isolation and cell culture

Mice were sacrificed at the age of 12 weeks by cervical dislocation. Islets were isolated by collagenase digestion as previously described (Gonzalez et al., 2013). In some experiments, islets were subjected to trypsin digestion to obtain isolated cells, and then cultured overnight at 37 °C in RPMI 1640 (Sigma, Madrid, Spain) supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, 0.1 mg/mL streptomycin and 11 mM D-glucose (Quesada et al., 2000).

2.4. Patch-clamp recordings

Electrophysiological measurements were performed from superficial β-cells in intact islets using an EPC-10 USB patch-clamp amplifier and the Patch Master Software suite (HEKA Elektronic, Lambrecht/Pfatz, Germany). Intact islets were hold by gentle suction applied to the interior of a wide-bore holding pipette as previously reported (Göpel et al., 1999). The perforated-patch configuration was used for the membrane potential recordings (Gonzalez et al., 2013). The pipette solution contained (in mM): 76 K_2SO_4, 10 NaCl, 10 KCl, 1 $MgCl_2$, 5 HEPES (pH = 7.35 with KOH), and 0.24 mg/mL of the pore-forming antibiotic amphotericin B; the bath solution contained (in mM): 140 NaCl, 3.6 KCl, 1.5 $CaCl_2$, 5 $NaHCO_3$, 0.5 $MgSO_4$, 0.5 NaH_2PO_4, 10 HEPES (pH = 7.4 with NaOH) and D-glucose as indicated. Exocytosis was monitored using the standard whole-cell configuration and recording cell capacitance changes through the sine +DC mode of the Lock-In amplifier included in the Patch Master software (Gonzalez et al., 2013). For these experiments, the pipette solution contained (in mM): 140 CsCl, 10 NaCl, 1 $MgCl_2$, 0.05 EGTA, 3 Mg-ATP, 0.1 cAMP and 5 HEPES (pH = 7.2 with CsOH), whereas the bath solution contained (in mM): 118 NaCl, 5.6 KCl, 20 tetraethylammonium–Cl, 1.2 $MgCl_2$, 5 $CaCl_2$, 5 HEPES and 5 D-glucose (pH = 7.4 with NaOH). Only experiments with stable and low access resistance and small leak currents were used. The seal resistance was typically >3 MΩ. All experiments were carried out at physiological temperature (34–36 °C). β-Cells were functionally identified by the ability to generate the characteristic oscillatory electrical activity in the presence of glucose and steady-state inactivation of Na^+ currents (Gonzalez et al., 2013; Göpel et al., 1999, 2000).

2.5. Intracellular Ca^{2+}, NAD(P)H and mitochondrial membrane potential measurements

Isolated islets were allowed to recover in the isolation medium for at least 2 hours at 37 °C and 5% CO_2 before experiments. For intracellular calcium ($[Ca^{2+}]_i$) recordings, islets were incubated for 1 h at room temperature with 2 μM fura-2 (for conventional fluorescence microscopy) or fluo-4 (for confocal microscopy). For intact islet Ca^{2+} signaling, recordings were performed under an inverted epifluorescence microscope (Axiovert 200; Zeiss, Jena, Germany) equipped with 360 and 380 nm band-pass filters. Recordings were expressed as the ratio of fluorescence at 360 and 380 (F360/380). Images were taken every 3 s. Intracellular $[Ca^{2+}]$ changes in response to stimuli were analyzed as previously described (Rafacho et al., 2010). For transient changes in $[Ca^{2+}]_i$, the basal fluorescence (F_0) was subtracted to the maximal fluorescence and expressed as ΔF ($F - F_0$). Additionally, as a measure of global $[Ca^{2+}]_i$ increase, the area under the curve (AUC) was calculated on the last 5 minutes of each glucose stimulus. Changes in NAD(P)H autofluorescence and mitochondrial membrane potential ($\Delta\Psi_m$) were monitored with the imaging system mentioned earlier (Rafacho et al., 2010). For NAD(P)H autofluorescence, a 365 nm band-pass filter was used, and emission was filtered at 445 ± 25 nm. Images were acquired every 60 s. For measurement of NAD(P)H in isolated cells, cells were cultured overnight in RPMI 1640. Recordings were plotted as the increase of fluorescence referred to the fluorescence in the basal condition. $\Delta\Psi_m$ was measured after loading fresh isolated islets for 10 minutes with 10 μM rhodamine-123 (Rhod-123). Images were taken every