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Sex differences in insulin resistance in GABAB1 knockout mice

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ABSTRACT

Aims: We have previously demonstrated that the absence of functional GABA B receptors (GABABRs) disturbs glucose homeostasis in GABAB1KO mice. The aim of this work was to extend our studies of these alterations in GABAB1KO mice and investigate the sexual differences therein.
Main methods: Male and female, GABAB1KO and WT mice were used. Glucose and insulin tolerance tests (GTT and ITT), and insulin and glucagon secretion tests (IST and GST) were performed. Blood glucose, serum insulin and hyperglycemic hormones were determined, and HOMA-IR calculated. Skeletal muscle insulin receptor β subunit (IRβ), insulin receptor substrates 1/2 (IRS1, IRS2) and hexokinase-II levels were determined by Western blot. Skeletal muscle insulin sensitivity was assessed by in vivo insulin-induced Akt phosphorylation (Western blot). Food intake and hypothalamic NPY mRNA expression (by qPCR) were also evaluated.
Key findings: Fasted insulin and HOMA-IR were augmented in GABAB1KO males, with no alterations in females. Areas under the curve (AUC) for GTT and ITT were increased in GABAB1KO mice of both genders, indicating compromised insulin sensitivity. No genotype differences were observed in IST, GST or in IRβ, IRS1, IRS2 and hexokinase-II expression. Akt activation was severely impaired in GABAB1KO males while no alterations were observed in females. GABAB1KO mice showed increased food intake and NPY expression.
Significance: Glucose metabolism and energy balance disruptions were more pronounced in GABAB1KO males, which develop peripheral insulin resistance probably due to augmented insulin secretion. Metabolic alterations in females were milder and possibly due to previously described reproductive disorders, such as persistent estrus.

Introduction

Type 2 diabetes (T2D) involves chronic dysregulation of glucose metabolism and impaired insulin sensitivity. More than 80% of patients progressing to T2D are hyperinsulinemic and insulin resistant (Pal, 2009). In T2D, defects in insulin-stimulated glucose uptake in skeletal muscle are major factors inducing impaired glucose homeostasis, including alterations in protein expression, enzyme activation/deactivation or sensitivity to endogenous ligands.

GABA, the main inhibitory neurotransmitter in the brain, is found at high concentrations in Langerhans islets (Franklin and Wollheim, 2004). Although a complete islet GABA system was demonstrated, the role of GABA in pancreatic physiology is less characterized. GABA inhibits high glucose-stimulated insulin secretion through GABAB receptors (GABABRs) in MIN6 cells and in rat/mouse islets (Bonaventura et al., 2012; Braun et al., 2004; Brice et al., 2002). We have recently described that GABAB agonists and antagonists alter glucose homeostasis in mice (Bonaventura et al., 2012). Previously we demonstrated that the absence of functional GABABRs in GABABR knockout mice (GABAB1KO) induced high pancreas insulin content and insulin resistance (Bonaventura et al., 2008).

Regarding insulin signaling, it binds to its receptor (IR), which is autophosphorylated and in turn phosphorylates IRS1 and IRS2. pIRS1/2 activate phosphoinsitol PI-3-kinase (PI3K) (Okada et al., 1994) which activates protein kinase B (PKB/Akt) (Franke et al., 1997). Akt is the major effector exerting the metabolic effects of insulin, including glucose transport, glycogen synthesis, fat deposition and protein synthesis. Loss of Akt signaling leads to glucose homeostasis impairment (Saltiel and Kahn, 2001).

In addition to insulin, other hormones involved in blood glucose control are glucagon, growth hormone (GH) and corticosterone. GH increases plasma glucose by decreasing peripheral glucose uptake. An

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increased GABAergic tone inhibits GH secretion (Tuomisto and Mannisto, 1985). Moreover, somatotropes express GABABRs (Mayerhofer et al., 2001) and stimulation of pituitary GABABRs increases GH secretion (Gamel-Didelon et al., 2002). Glucagon is secreted by α-cells in response to low blood glucose and its secretion is inhibited by insulin. Wendt et al. (Wendt et al., 2004) demonstrated that GABA released from β-cells inhibits glucagon release from α-cells in rat pancreas, confirming results in mice (Gilon et al., 1991). Others proposed that insulin sensitized α-cells to β-cell-secreted GABA (Xu et al., 2006). Cortisol increases blood glucose by inhibiting glucose uptake and utilization; it also stimulates appetite and changes fat metabolism (van Raalte et al., 2009). As GABA, through GABABRs, alters the corticotropic axis (Marques and Franci, 2008), it could modulate the hyperglycemic effects of cortisol.

GABA is also considered an orexigenic stimulus (King, 2006), and a role for central GABABRs controlling food intake has been suggested (Ebenezer and Prabhaker, 2007). Neuropeptide Y (NPY), is expressed in the arcuate nucleus, and is a potent stimulant of food intake. Evidences suggest that the anorexigenic effects of insulin are exerted by inhibition of NPY in the arcuate, acting through GABAARs and GABABRs present in NPY neurons (Sato et al., 2005).

Therefore, here we evaluated how the lack of functional GABABRs affects different targets participating in blood glucose control in GABAB1KO mice and the sexual differences therein.

Materials and methods

Animals

GABAB1KO mice, generated in the BALB/C inbred strain (Schuler et al., 2001), were obtained by intercrossing heterozygous animals and the day of birth was recorded. Mice were genotyped by PCR analysis, as described previously (Catalano et al., 2005). Animals were fed ad libitum. All studies were performed according to protocols for animal use, approved by the Institutional Animal Care and Use Committee (IBYME-CONICET) that follows the NIH guidelines. Adult 2–3 month-old female and male WT and GABAB1KO mice were used. For each experimental design animals were age-matched littermates. Animals were sacrificed by decapitation in minimal conditions of stress.

Basal blood glucose titers and glucose tolerance test

Blood glucose was measured by a One touch® Ultra™ glucose meter (Lifescan, Scotland Ltd, strips were kindly donated by Johnson & Johnson, Argentina) from tail blood. The precision for this method is 2.0 and 1.6% for low and high glucose titers respectively (range 70–360 mg/dl). For the glucose tolerance test (GTT) intraperitoneal (ip) glucose (3 g/kg body weight (BW)) (Bonaventura et al., 2008) was injected to uniformly overnight fasted mice of all groups (15–18 h) and blood glucose was evaluated at 0, 30, 60 and 75 min post glucose administration. Results were informed as Area under the curve (AUC).

Insulin determination and insulin secretion test (IST)

Serum insulin was measured with an Ultrasensitive insulin mouse ELISA kit (Chrystalchem, Chicago, Il) at 0, 10, 20, 30 and 60 min after the ip glucose injection of 3 g/kg BW in mice fasted for 15–18 h in samples taken during the GTT. Results were informed as AUC.

HOMA index calculation

HOMA of insulin resistance (HOMA-IR) was calculated with basal blood glucose and basal insulin measured after overnight fasting, as previously described (Bonaventura et al., 2012). HOMA-IR = Fasting insulin (μU/ml) × Fasting glucose (mmol/L)/22.5.

Insulin tolerance test (ITT)

Blood glucose was measured as above in 2–4 h fasted mice after 0, 10, 20, 30 and 60 min of an ip injection of 1 U/kg BW of porcine humanized insulin (a gift from Laboratorios Beta, Buenos Aires, Argentina). Results were informed as AUC.

Glucagon secretion test (GST)

3–4 h fasted animals were injected with insulin (1U/kg i.p.) and blood was collected from tail at 0 and 30 min post-injection (Zhou et al., 2004). Glucagon was determined by RIA (Glucagon RIA KIT, Millipore, MA) according to the manufacturer protocol. Results were informed as AUC.

Basal hyperglycemic serum hormones determinations

Glucagon was determined by RIA, as above. For corticosterone determination, serum samples were extracted with dichloromethane and corticosterone content determined by RIA, as previously described (Bonaventura et al., 2012; Repetto et al., 2010). Growth hormone (GH) was also determined by RIA, as previously described (Catalano et al., 2005).

In vivo peripheral tissue response to insulin

Fasted animals (2–4 hr) were anesthetized with avertin 2% (12 ml/kg i.p.). The abdominal cavity was opened and 2 U/kg of insulin was injected (in 0.5 ml saline) into the portal vein. At time points 0, 1 and 5 min post-injection, portions of skeletal muscle were excised and flash frozen in liquid N_2 and stored at −70 °C until used. Tissues were disrupted in 10 volumes of lysis buffer (1% SDS, NaCl, 10 mM EDTA, Tris–HCl) containing phosphatases and proteases inhibitors (LiCl, Na_3VO_4, PMSF, ZPCK, TAME, TPLC, β-glicerolphosphate) at 4 °C with a Polytron homogenizer, samples were centrifuged at 10000 rpm and pellets discarded. Supernatants were kept frozen until used.

Western blot analysis

Western blot analysis for IRS1, IRS2, IRβ subunit, hexokinase-II, pAkt, Akt, and actin were performed in skeletal muscle homogenates of WT and GABAB1KO of both genders. 50 μg of proteins and biotinylated molecular weight markers were subjected to 8% SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were blocked with 5% non-fat milk in 0.5% Tween PBS (PBS-T). For IRS-1, IRS-2 and IRβ membranes were incubated over night (ON) at 4 °C with first antibody diluted in PBS-T, 5% non-fat milk (Millipore, CA, IRS1, cat # 06–248: 1:500, IRS2, cat # 06–506: 1:500; IRβ, cat # 07–724: 1:125) followed by 2 hr incubating with HRP conjugated anti-rabbit (Vector, CA, cat # PI-1000: 1:3000) and anti-biotin (Cell Signalling, MA, cat # SP-3010: 1:4000) in PBS-T, 1% BSA, at room temperature (RT). For Hexokinase-II, membranes were incubated ON at 4 °C with first antibody diluted in PBS-T, BSA 1% (Cell Signalling, cat # 2867: 1:500) followed by incubation with second antibody as described above. Membranes were stripped with stripping buffer (Tris–HCl 62,5 mM, SDS 2%, β-mercaptoethanol 100 mM, pH = 6.7) and re-used for actin determination: membranes were incubated 1 h at RT with first antibody diluted in PBS-T, BSA 2% (Sigma, MI, cat # CP01: 1:5000) followed by incubation with HRP conjugated anti-mouse (Vector, CA, cat # PI-2000: 1:3000).

For p-Akt, membranes were incubated ON at 4 °C with first antibody diluted in PBS-T, BSA 5% (Cell Signalling, MA, cat # 9271: 1:1000) followed by incubation with second antibody. Membranes were stripped and re-used for Akt determination: membranes were incubated 1 h with first antibody diluted in PBS-T, 1% BSA (Santa Cruz Biotechnology Inc., CA, cat # sc-1618: 1:500) followed by 1 h incubation with HRP conjugated anti-goat (Santa Cruz Biotechnology Inc., CA, cat # sc-2953: 1:4000).