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Pancreatic islets isolated from β_2 adrenergic receptor knockout mice show reduced insulin secretion in response to nutrients

Anderson Carlos Marçal[1*], Ana Paula Couto Davel[2], Angelo Rafael Carpinelli[3], Patrícia Chakur Brum[4], Luciana Venturini Rossoni[3] and Carla Roberta de Oliveira Carvalho[3]

*[1]Departamento de Morfologia, Universidade Federal de Sergipe, Av. Marechal Rondon, s/n, 49100-000, São Cristóvão, Sergipe, Brazil. [2]Departamento de Biologia Estrutural e Funcional, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil. [3]Departamento de Fisiologia e Biofísica, Instituto de Ciências Biomédicas I, Universidade de São Paulo, São Paulo, São Paulo, Brazil. [4]Escola de Educação Física e Esporte, Universidade de São Paulo, São Paulo, São Paulo, Brazil. *Author for correspondence. E-mail: acmarcal@yahoo.com.br*

ABSTRACT. Activation of β_2 adrenergic receptors by catecholamine or catecholamine-mimetic substances may enhance insulin secretion. We herein investigated KCl- and nutrient-stimulated insulin secretion in pancreatic islets isolated from β_2 knockout (β_2KO) mice. β_2KO mice showed reduced body weight, fasting hypoglycaemia associate to a similar fasting insulinemia compared to control. β_2KO mice also showed reduced glucose tolerance despite the higher sensitivity to insulin. Glucose-induced insulin secretion was impaired in pancreatic islets isolated from β_2KO mice. Leucine-induced (20mM) insulin secretion was diminished in pancreatic islets isolated from β_2KO mice when compared to control one. The depolarizing effect of KCl on insulin secretion was also impaired in pancreatic islets from β_2KO mice. These results suggested a possible role of β_2 adrenergic receptors on nutrient-induced insulin secretion.

Keywords: adrenergic receptors, pancreatic beta cells, glucose, leucine.

Ilhotas pancreáticas isoladas de camundongos com deleção do receptor adrenérgico β_2 apresenta reduzida secreção de insulina em resposta a nutrientes

RESUMO. A ativação dos receptores β_2-adrenérgicos por catecolaminas ou miméticos a catecolaminas podem aumentar a secreção de insulina. Nós investigamos a secreção de insulina estimulada por nutrients e KCl em ilhotas pancreáticas isoladas de camundongos com deleção dos receptores β_2-adrenérgicos (β_2KO). Camudongos β_2KO apresentaram reduzido peso corporal, hipoglicemia de jejum associada a semelhante concentração de insulina plasmática de jejum comparada ao grupo controle. Camundongos β_2KO apesar de apresentarem aumento da sensibilidade a insulina também apresentaram reduzida tolerância a glicose. A secreção de insulina induzida com glicose foi alterada em ilhotas pancreáticas isoladas de camundongos β_2KO. Secreção de insulina induzida por leucina (20mM) foi diminuída em ilhotas pancreáticas isoladas de camundongos β_2KO quando comparado ao controle. O efeito despolarizante do KCl sobre a secreção de insulina também foi alterado em ilhotas pancreáticas de camundongos β_2KO. Estes resultados sugerem um possível papel dos receptores β_2-adrenérgicos na secreção de insulina induzida por nutrientes.

Palavras-chave: receptores adrenérgicos, células beta pancreáticas, glicose, leucina.

Introduction

D-glucose is the main physiological stimulus for insulin-secretion. After being transported into β-cells by GLUT-2, glucose is phosphorylated by a glucokinase and subsequently metabolized through the glycolytic pathway. When glucose concentration is elevated (above 7 mM), the ATP/ADP ratio also increases, leading to the closure of the ATP-sensitive K^+ channel (K_{ATP} channel). This event ends up depolarizing the plasma membrane and opening the voltage-sensitive Ca^{2+} channels, which in turn increases the intracellular concentration of calcium and causes the exocytosis of insulin granules into the bloodstream (MACDONALD et al., 2005). Amino acids (AA) such as leucine and arginine are insulin secretion inducers when the serum glucose concentration is high. AA depolarize the plasma membrane (by closing the K_{ATP} channel) and increase calcium content in pancreatic beta cells (HENQUIN et al., 2003).

The sympathetic nervous system is able to modulate insulin secretion (NARIMIYA et al., 1981; MARÇAL et al., 2006; BRUM et al., 2006). There are three recognized subtypes of adrenergic beta receptors (β_1, β_2, β_3) that can mediate the enhancement of insulin and glucagon secretion when activated by adrenalin,

noradrenalin or adrenomimetics (NARIMIYA et al., 1981; MARÇAL et al., 2006; AHRÉN, 2000). These receptors are also involved in several metabolic processes such as activation of hepatic gluconeogenesis (FISHER et al., 1986), lipolysis (BERLAN; DANG TRAN, 1978) and subtype-4 glucose transporter (GLUT4) activity (ZANQUETTA et al., 2006).

Mice with total deletion of beta adrenoceptors showed glucose intolerance with increased sensitivity to insulin, indicating a possible alteration in insulin secretion (ASENSIO et al., 2005). However, the subtype of adrenergic receptor involved is still unknown. In this regard, we used β_2KO mice to investigate the impact of β_2 adrenergic receptor on whole-body glucose homeostasis and insulin secretion on pancreatic islets.

Pancreatic islets from β_2KO mice displayed reduced insulin secretion when incubated with glucose, amino acids or KCl. These results suggest that the β_2 adrenergic receptors play a key role in the nutrient-induced insulin secretion.

Material and methods

Animals

Heterozygote β_2-AR +/- mice were mated to generate both homozygote β_2-AR +/+ and -/- mice. Several matings were carried out to obtain knockout mice for the β_2-AR -/- (β_2KO) (CHRUSCINSKI et al., 1999; MEDEIROS et al., 2008). Four-month-old mice were used. Each group of five animals was kept in cages under controlled temperature (23 ± 2°C) and light (12h light/dark cycle) conditions. The animals were given a commercial chow (Nuvital, Brazil) and water ad libitum. All experimental protocols were approved by the Ethics Committee on Animal Experimentation of the Institute of Biomedical Sciences of the University of São Paulo, São Paulo State, Brazil.

Chemicals

Rat insulin labeled with ^{125}I was obtained from Amershan Pharmacia (São Paulo, SP, Brazil). All other reagents were obtained from Sigma, unless otherwise mentioned. Insulin antibody was a gift from Dr. Leclercq-Meyer, Université Libre de Bruxelles, Belgium.

Glucose tolerance test (gTT)

Mice were fasted overnight (12 hours) and their blood was collected by puncturing the tail vein at 0, 15, 30, 60, 90 and 120 min. after a bolus injection of glucose (2 g kg^{-1} body weight of 20% D-glucose solution) diluted in saline solution (0.9%). Glycaemia was determined using the glucometer (Roche®, Mannheim, Germany).

Insulin tolerance test (iTT) and the percentage of periepididymal fat pad

After a 4-hour fast, animals were anesthetized with thiopental (40 mg kg^{-1} body weight). Insulin diluted in saline solution (0.9%) was then immediately intraperitoneally injected (25 mU g^{-1} body weight) and blood samples were collected from the tail at 0, 5, 10, 15, 20, 25, 30, 40, 60 and 120 min. after injection for glycaemia determination. Subsequently, these same mice were decapitated, and then, abdominal cavity of animals from both groups were open, periepididymal fat pads were collected and weighed to determine epididymal fat pad content (% periepididymal fat pad = g g^{-1} body weight × 100).

Isolation of the pancreatic islets

Pancreatic islets were obtained from mice as previously described (LATORRACA et al., 1999) with few modifications. The pancreas was inflated with Hanks solution containing type V collagenase (35 mg mL^{-1}), kept at 37°C for 20 min. in a water bath and stirred for an additional 1 min. They were then washed with a Krebs-Henseleit buffer solution containing 115 mM NaCl, 5 mM KCl, 24 mM $NaHCO_3$, 1 mM $CaCl_2$ and 1 mM KCl_2. The islets were then collected using a magnifying glass.

Incubation of the pancreatic islets

A group of five islets was transferred to plates containing 1 mL Krebs-Henseleit buffer solution supplemented with 0.125% albumin for 60 minutes at 37°C in the presence of glucose (5.6 mM). This solution was bubbled with a mixture of O_2 (95%) and CO_2 (5%). After the pre-incubation period, the incubation with different glucose concentrations (2.8, 5.6, 8.3, 11.1 and 16.7 mM) took place for an additional 60 min. Another group of islets was homogenized and the total insulin content was determined by radioimmunoassay (RIA). For the tests carried out with amino acids, pancreatic islets were incubated with L-leucine (20 mM) and/or L-arginine (20 mM) with or without glucose (8.3 mM), as previously described (LATORRACA et al., 1999). Furthermore, incubation with a high concentration of KCl (30 mM) was carried out in the presence of glucose (8.3 mM). At the end of incubation, insulin was measured by RIA. Aliquots were collected from the incubated pancreatic islets and plasma insulin concentrations were measured by RIA

using rat insulin marked with ^{125}I from Amershan Pharmacia (São Paulo, São Paulo, Brazil).

Statistical analysis

The results obtained with both mice strains were presented as mean ± SEM. The data were analyzed using Student's t-test for two conditions and a two-way ANOVA test with Bonferroni post hoc test for more than two conditions, and then systematized by GraphPad Prism, 4.00 for Windows (GRAPHPAD PRISM, 2003).

Results and discussion

Body weight, epididymal fat pad content, fasting blood glucose and insulin levels of 4-month-old β_2KO and wild-type mice were measured. β_2KO mice presented both reduced body weight and perigonadal fat compared to wild-type mice (Table 1). Fasting blood glucose levels were 26% lower in β_2KO animals ($p < 0.05$). No difference was detected between the groups for plasma insulin levels. However, HOMA was enhanced in β_2KO mice when compared to controls (Table 1).

Table 1. Body weight, visceral adipose tissue, blood glucose level and serum insulin level of wild-type and β_2KO mice.

	Wild-type	β_2KO
Body weight (g)	34 ± 1.5 (10)	29.1 ± 1.2* (10)
% Periepididymal fat pad (g g^{-1} body weight x 100)	1.3 ± 0.122 (10)	1,01 ± 0.073* (10)
Glycaemia (mM)	9.6 ± 0.31 (09)	7.1 ± 0.25* (09)
Insulinaemia (μU mL^{-1})	21.3 ± 2.4 (09)	28.1 ± 4.8 (09)
HOMA	9.1 ± 0.05 (09)	8.8 ± 0.04* (09)

The results are representative of 5 distinct experiments and are described as mean +/- SEM. The asterisk, *, indicates $p < 0.05$. The number of animals is indicated in brackets.

Glucose overload in the gTT causes a more pronounced increase in blood glucose levels in β_2KO mice at 15 (125%) and 20 (134%) min compared to control (Figure 1A). As expected, insulin infusion induced a reduction in blood glucose levels in both wild-type and knockout mice. However, the magnitude of this effect was greater in β_2KO mice at the 15, 20 and 25 min time points after insulin injection by 20, 36, and 33%, $p < 0.01$, respectively (Figure 1B). AUC-glucose was significantly diminished in the β_2KO mice compared to control, $p < 0.01$ (Figure C insert at Figure 1B). These results were corroborated by the HOMA test (Table 1).

Reduced insulin secretion in response to glucose stimulus was observed in pancreatic isolated islets from β_2KO mice, as indicated by the increased EC_{50} of glucose stimulus compared to wild-type mice; EC_{50} of 11.4 and 8.1 mM, $p < 0.05$ (Figure 2).

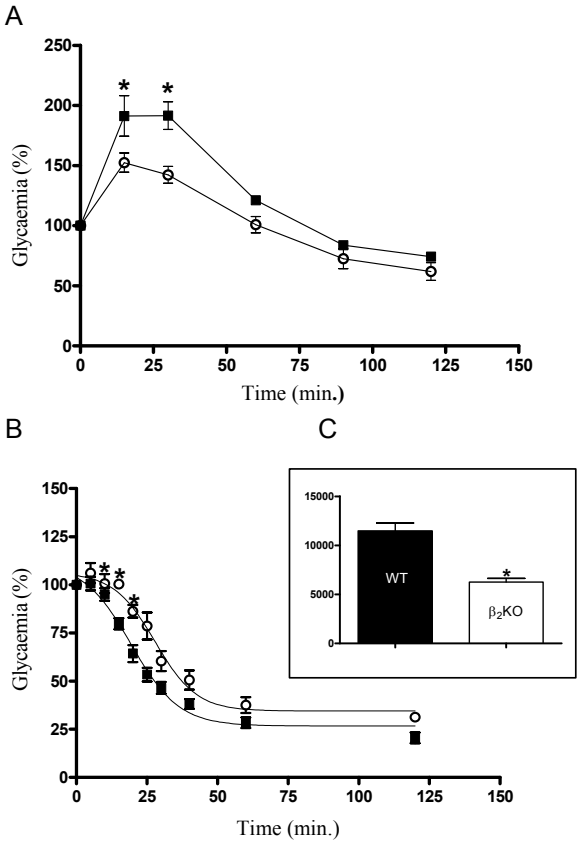

Figure 1. β_2KO mice had altered glucose tolerance test (A) with increased insulin sensitivity (B). The data are expressed as percentage of the basal blood glucose level (initial time (t = 0)) of each β_2KO (open circle) and wild-type (black square) mice. The AUC-glucose represents the values obtained during insulin sensitivity test (insert on Figure 1B). The analysis was performed with 4 animals of each strain. A two-way ANOVA test followed by a Bonferroni test was run. *$p < 0.01$.

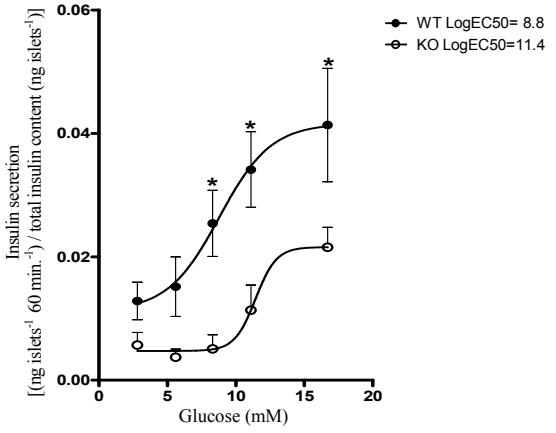

Figure 2. Static insulin secretion in groups of 5 isolated pancreatic islets incubated with 2.8, 5.6, 8.3, 11.1 and 16.7 mM glucose for 60 min. The data are expressed as means ± SEM by total insulin content (ng $islets^{-1}$) of 7 distinct experiments with triplicate. A two-way ANOVA test followed by a Bonferroni test was run. *$p < 0.05$.

Although leucine is able to enhance glucose-induced insulin secretion, this effect was abolished in

pancreatic islets isolated from the β_2KO mice (Figure 3), however, this response was not observed in pancreatic islets incubated only with arginine (Figure 3).

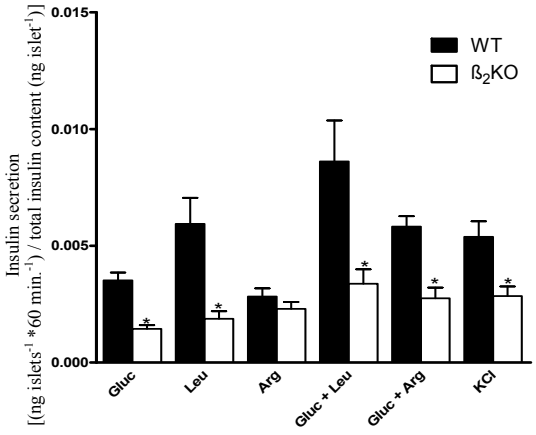

Figure 3. Insulin release by isolated islets in response to nutrients: Gluc (8.3 mmol L^{-1}), Leu (20 mmol L^{-1}), Arg (20 mmol L^{-1}), Gluc+Leu and Gluc+Arg, KCl (30 mM), same concentrations as for the individual stimuli. The data are expressed as means ± SEM by total insulin content (ng $islets^{-1}$) of 7 experiments with triplicate. *$p < 0.05$.

The insulin secretion induced by the hyperpolarizing agent KCl was also reduced to 43% of that detected in the isolated pancreatic islets of the wild-type (Figure 3). Furthermore, the total insulin content was reduced in pancreatic islets β_2KO mice as compared to the control group (WT = 125.2 ± 5.6 vs β_2KO = 102.8 ± 8.5 ng $islets^{-1}$, $p < 0.05$) (Figure 4).

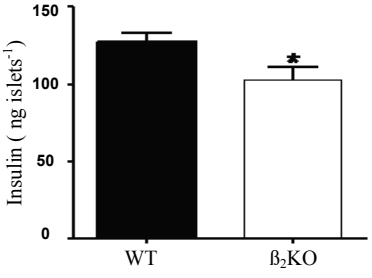

Figure 4. Insulin content in isolated pancreatic islets from wild-type (dark bar) and β_2KO (open bar) mice. The data are expressed as means ± SEM of 7 mice of each strain with triplicate. *$p < 0.05$.

β_2KO mice showed lower body weight, as reported by Chruscinski et al. (1999). Those animals are leaner when compared to the control group, and that result was evidenced by the reduced weight of the periepididymal fat pad. The amount of perigonadal fat is considered a predictor of obesity in rodents according to Rogers and Webb (1980).

β-adrenergic stimulation inhibits insulin-stimulated glucose uptake in skeletal muscle and in white adipose tissue. The epinephrine decreases insulin-stimulated glucose transport in skeletal muscle from both rats and humans, and in rat adipose cells through reduction of GLUT4 translocation to the plasma membrane as demonstrated by Han and Bonen (1998), Bonen et al. (1992), Jones and Dohm (1997), Laurent et al. (1998), Nishimura et al. (1991), and Yang et al. (2002). However, White and Kahn (1994), and Saltiel and Kahn (2001) showed that insulin is the most important extracellular signaling agent that leads to glucose uptake. Alterations in the intracellular insulin signaling are associated with the development of insulin resistance and DM2 according to Carvalho et al. (1999), Marçal et al., (2012), and Yamauchi et al. (1996). Phosphorylation of both insulin receptor and insulin receptor substrates, IRS-1 and IRS-2 is reduced when the beta adrenergic receptor is activated as well demonstrated by Doronin et al. (2002). In this regard, the improvement in insulin sensitivity reported herein in β_2KO mice is in agreement with the established effects of catecholamines on intermediary metabolism.

Miller (1981) described that pancreatic islets have an abundant sympathetic innervation. According to this author, Peterhoff et al. (2003) described that the α_2-adrenergic receptors when activated by epinephrine or adrenomimetics, causes inhibition of insulin secretion. On the other hand, β_2-adrenergic receptors have a dual effect, stimulating or inhibiting insulin secretion as well as pointed out by Narimiya et al. (1981), Marçal et al. (2006), Ahrén and Lundquist (1981), and Kurose et al. (1990). The reduced glucose-stimulated insulin secretion (GSIS) observed in β_2KO mice could be associated with a prolonged activation of α-adrenergic receptors as demonstrated by Sjoholm (1991). Another interesting observation was the reduced effect of glucose plus arginine and leucine on insulin secretion. Despite the fact that the mechanism of L-arginine-induced insulin secretion is not fully clarified, many authors suggest that the potentiation of glucose-induced insulin secretion by L-arginine is mediated by β-cell membrane depolarization due to the electrogenic influx of the cationic amino acid into the β-cell as described by Charles et al. (1982), Henquin and Meissner (1981), Blachier et al. (1989), Hermans et al. (1987), and Smith et al. (1997). Thus, amino acid depolarizes the plasma membrane, whose effect is enhanced by glucose as shown by Hermans et al. (1987), and stimulates Ca^{2+} influx by activation of voltage-sensitive Ca^{2+} channels according to Hermans et al. (1987), Smith et al. (1997), and Weinhaus et al. (1997).

According to the findings from Sener and Malaisse, (1980), Gylfe (1976), Panten et al. (1972), and Carpinelli and Malaisse (1981), L-Leucine induces insulin secretion by two mechanisms: (i) enhanced mitochondrial metabolic activity through activation of

glutamate dehydrogenase, and (ii) by transamination to α-ketoisocaproate and subsequent entry into the TCA cycle via acetyl-CoA leading to an increase in ATP production. Alteration of insulin secretion in pancreatic islets from β_2KO mice could be related to a reduction in the uptake and/or metabolism of these nutrients.

The electrical activity of beta cell membrane, through Ca^{2+}-dependent action potentials, plays a key role for insulin secretion: the secretion of insulin is abolished in the absence of calcium in agreement with MacDonald et al. (2005), Henquin et al. (2003), Carpinelli and Malaisse (1981), and Ashcroft (2005). However, change in function and/or expression of voltage-gated Ca^{2+} channels cannot be ruled out in isolated pancreatic islets from β_2KO mice.

Conclusion

We have demonstrated herein that β_2-adrenoceptor deletion induces remarkable modifications of glucose-stimulated insulin secretion in isolated pancreatic islets. The depolarization of beta cells plasma membrane is involved in the effect and deserves to be further investigated.

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