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Beta-adrenergic blockade increases GLUT4 and improves glycemic control in insulin-treated diabetic Wistar rats

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ABSTRACT

Objective: Unequivocal modulation of glycemic homeostasis by chronic beta-adrenergic blockade in diabetes has never been demonstrated. This study investigates the participation of beta-adrenergic system in glycemic control and muscle glucose transporter GLUT4 expression in insulin-treated diabetic rats.
Methods: Insulin-treated diabetic Wistar (W) or spontaneously hypertensive rats (SHR) were additionally treated with propranolol, and glycemic homeostasis and expression of some target mRNAs and proteins in soleus and extensor digitorum longus (EDL) muscles were analyzed.
Results: Insulin improved glycemic control in both strains. Importantly, in W, propranolol promoted a further improvement in glycemic control, which was accompanied by decreased PKA and *Tnf* expression, and increased *Slc2a4* and GLUT4 in EDL. Those effects were not observed in diabetic-SHR.
Discussion: Propranolol-induced decrease in beta-adrenergic activity in skeletal muscles of insulin-treated diabetic Wistar rats increases GLUT4 expression in EDL, improving glycemic control. These outcomes represent a positive effect of nonselective beta-blockade, which might be extended to autonomic neuropathy.

1. Introduction

Diabetes mellitus is a syndrome of multiple etiologies, due to the impaired insulin secretion and/or insulin action, resulting in the presence of hyperglycemia, which involves both increased hepatic glucose production and decreased peripheral (primarily muscle) glucose uptake (DeFronzo, 2004).

Hypertension frequently coexists with diabetes, and insulin resistance has been postulated to be involved in the pathogenesis of this association (Morisco et al., 2006; Reaven, 2006). Hyperinsulinemia stimulates peripheral sympathetic activity in healthy subjects (Berne et al., 1992), an effect that is not impaired in insulin resistant states; thus explaining the hypertension development. In fact, hypertensive or obese insulin resistant subjects show high basal sympathetic activity (Tentolouris et al., 2008).

Beta-adrenergic agonists classically enhance glucose uptake (Chiasson et al., 1981; Sato et al., 2014); however, that effect changes according to either the presence of insulin (Chiasson et al., 1981) or chronic conditions (Hunt et al., 2002). Differently, beta-adrenergic blockade seems to improve glucose uptake both acutely (Chiasson et al., 1981) and chronically (Haenni and Lithell, 1994); pointing out how these regulations are complex. In diabetes, an autonomic nervous system dysbalance may be expected as consequence of autonomic neuropathy or beta-blocking treatment; however, whether such conditions affect glycemic control has not been clearly reported.

Autonomic neuropathy has only been related to acute impairment of hypoglycemia recovery in patients under insulin therapy (Cryer, 1994; Gerich, 1988); and its repercussion on ordinary glycemic control is still completely unknown. Similarly, beta-blocker treatment has often been withheld from diabetic patients, but there is no convincing report that it degenerates glycemic control. It has been proposed that beta-blockers impair glycemic recovery after hypoglycemia in type 1 diabetes (Lager, 1983; Ryan et al., 1985), by reducing adrenergic-induced glucose hepatic efflux; but that was only acutely demonstrated (Shamoon and Sherwin, 1984), and not consistently observed (Kleinbaum and Shamoon, 1984). In type 2 diabetes, chronic treatment with propranolol has been proposed to have no effect on glycemic control

Abbreviations: ADRB2, beta 2-adrenergic receptor; cAMP, cyclic adenosine monophosphate; EDL, extensor digitorum longus; GLUT4, glucose transporter 4; NFKB, nuclear factor-κB; PKA, cAMP-dependent protein kinase; *Slc2a4*, solute carrier family 2 member 4; TNF, tumor necrosis factor-α; WV, vehicle-treated diabetic Wistar; WI, insulin-treated diabetic Wistar; WIP, insulin + propranolol-treated diabetic Wistar; SV, vehicle-treated diabetic SHR; SI, insulin-treated diabetic SHR; SIP, insulin + propranolol-treated diabetic SHR.

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(Chellingsworth et al., 1989; Dornhorst et al., 1985; Wright et al., 1979), or to deteriorate glycemic control (Chellingsworth et al., 1989; Sawicki and Siebenhofer, 2001; Waal-Manning, 1976); the latter by reducing insulin secretion (Waal-Manning, 1976), which was not confirmed in other studies (Chellingsworth et al., 1989; Wright et al., 1979). Additionally, beta-blockade does not contribute to increasing the risk of hypoglycemia, even among patients prone to hypoglycemia (Sawicki and Siebenhofer, 2001). Hence, despite the fact that propranolol might increase the risk of new-onset diabetes (Bangalore et al., 2007; Gupta et al., 2008), the true effect of nonselective beta-blockade upon diurnal glycemic control, if any, has not been demonstrated yet, especially in insulin-treated subjects.

Glucose transporter 4 (GLUT4), encoded by *Slc2a4* gene, ensures adequate insulin-induced glucose clearance, being critical to glycemic homeostasis. Impaired GLUT4 expression in skeletal muscle has been related to insulin resistance (Corrêa-Giannella and Machado, 2013); and diabetes is widely shown to decrease *Slc2a4* mRNA and GLUT4 protein expression in skeletal muscles and white adipose tissue (Alves-Wagner et al., 2014; Mora and Pessin, 2000; Okamoto et al., 2011). Sympathetic nervous system has been proposed to modulate glucose metabolism on skeletal muscle (Nonogaki, 2000), which may involve changes in *Slc2a4*/GLUT4 expression. Regarding that, a previous study in spontaneously hypertensive rats (SHR), which display high sympathetic activity, has proposed a blurred regulation of *Slc2a4* mRNA and GLUT4 protein in a glycolytic/oxidative mixed skeletal muscle (Katayama et al., 1997). Recently, our group has demonstrated that *Slc2a4* mRNA is increased in soleus (oxidative) and extensor digitorum longus (EDL, glycolytic) muscles of SHR (Alves-Wagner et al., 2014), and when these animals were rendered diabetic, no decrease in *Slc2a4*/GLUT4 expression was observed. Thus, sympathetic activity displays an enhancer effect on *Slc2a4*/GLUT4 expression. Besides, we have reported that beta-adrenergic sympathetic activity preserves the GLUT4 expression in glycolytic muscle of non-hypertensive rats during fasting (Alves-Wagner et al., 2009), reinforcing a positive effect of beta-adrenergic activity on *Slc2a4*/GLUT4 regulation.

Skeletal muscle predominantly expresses the beta 2 subtype of beta-adrenergic receptor (Kim et al., 1991), which mediates adrenergic effects via the cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) pathway (Lynch and Ryall, 2008). Thus, whether sympathetic activity regulates the *Slc2a4*/GLUT4 expression, the cAMP/PKA pathway should be involved. Furthermore, increased cAMP/PKA activity in muscle has been shown to reduce the expression of the tumor necrosis factor (TNF, former TNF-α) (Lira et al., 2007; Saghizadeh et al., 1996), a potent repressor of *Slc2a4* gene (Furuya et al., 2013; Moraes et al., 2014); and that might be involved in the sympathetic activity-mediated regulation of *Slc2a4*/GLUT4 expression in diabetes.

Although beta-adrenergic effect upon *Slc2a4*/GLUT4 expression has been proposed in physiological conditions, nothing is known about this regulation in insulin-treated diabetes, pointing out that in diabetes decreased sympathetic activity may occur because of beta-blocker treatment and/or autonomic neuropathy development. Whether changes in sympathetic activity may alter the *Slc2a4*/GLUT4 expression, these conditions should modulate the glycemic homeostasis in diabetes. Thus, the purpose of the present study was to investigate glycemic homeostasis and the *Slc2a4*/GLUT4 expression in skeletal muscles of diabetic Wistar and SHR, subjected or not to the propranolol-induced beta-blockade.

2. Materials and methods

2.1. Chemicals

NPH insulin Humulin was from Eli Lilly and Company (Indianapolis, USA). Sodium thiopental was from Cristalia (Itapira, Brazil). Propranolol (P0884-DL-propranolol) was from Sigma-Aldrich (St. Louis, USA). Anti-GLUT4 (07-1404 — polyclonal antibody) was from Millipore (Temecula, USA), anti-beta2-adrenoceptor (SC569, rabbit polyclonal IgG) was from Santa Cruz Biotechnology (Santa Cruz, USA), anti-cAMP Protein Kinase Catalytic subunit (anti-PKA, ab26322 polyclonal) was from Abcam (Cambridge, USA), and rabbit anti-goat IgG was from Jackson ImmunoResearch (Pennsylvania, USA). Primers were from Eurofins MWG Operon (Ebersberg, Germany) and Integrated DNA Technologies (Iowa, USA).

2.2. Animals

Male Wistar rats (W) and spontaneously hypertensive rats (SHR) from the Animal Center of the Institute of Biomedical Sciences, University of São Paulo (São Paulo, Brazil), were rendered diabetic by intravenous injection of alloxan (40 mg/kg bw) at twelve weeks of age, as previously described (Sabino-Silva et al., 2010; Vestri et al., 2001). After 4 weeks of diabetes induction, animals were treated for one week with: vehicle, insulin (6 U/day, subcutaneously, 2 U at 8:00 AM and 4 U at 5:00 PM) or insulin + propranolol (16 mg/kg body weight/day, intraperitoneally, divided into 2 doses at 7:00 AM and 7:00 PM), as previously described (Freitas et al., 2005; Kimura et al., 1993). Vehicle-treated rats received both subcutaneous saline injections and intraperitoneal citrate injections; respectively, at the same moment of insulin and propranolol injections, in equal volumes. The groups were designed as follows: diabetic Wistar rats treated with vehicle (WV), or insulin (WI), or insulin plus propranolol (WIP); and diabetic SHR treated with vehicle (SV), or insulin (SI), or insulin plus propranolol (SIP). At the end of experiments, euthanasia was performed by high dose of pentobarbital. The experimental protocol (#015/2008) was approved by the Ethical Committee for Animal Research of the Institute of Biomedical Sciences, University of Sao Paulo.

2.3. Blood and urine collection and analysis

Twenty-four hour urine was collected before and after the treatment. For urine collection, animals were placed in metabolic cages from 9:00 AM to 9:00 AM. At the end of treatments, immediately after the 24-hour period in the metabolic cage, animals were anesthetized (sodium pentobarbital 40 mg/kg body weight), and blood (from the inferior vena cava) and skeletal muscles were collected. Plasma was used to measure glucose and insulin concentrations as previously described (Alves-Wagner et al., 2014), pointing out the animals were not subjected to previous food restriction.

2.4. Blood pressure analysis

In order to confirm the effectiveness of propranolol treatment, systolic blood pressure of a group of diabetic hypertensive rats was measured (by the tail-cuff method, LE 5001; Panlab S.I. Barcelona, Spain). One-week treatment with propranolol reduced systolic blood pressure from 176.1 ± 1.8 to 159.3 ± 1.8 mm Hg ($n = 7$ and 5, respectively; $P < 0.001$, Student t test).

2.5. Tissue collection and molecular analysis

Soleus and extensor digitorum longus (EDL) were collected at the end of treatment, under anesthetic condition. *Slc2a4* mRNA expression was analyzed by Northern blotting, and GLUT4 and beta2-adrenoceptors expression were analyzed by Western blotting (Alves-Wagner et al., 2009; Seraphim et al., 2007). Tumor necrosis factor (*Tnf*) mRNA was analyzed by RT-PCR as previously described (Freitas et al., 2009). PCR primers for *Tnf* (forward 5′-CGTCAGCCGATT TGCCATTTC-3′, reverse 5′-TGGGCTCATACCAGGGCTTGAG-3′) amplified an 116 pb cDNA fragment with a TM of 59 °C; and for *Gapdh* (forward 5′-ACATCATCCCTGCATCCACT-3′, reverse 5′-GGGAGTTGCTGTTGAAGT CA-3′) amplified a 258 pb cDNA fragment with a TM of 55.7 °C.