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Research Paper

Chronic blockade of the AT2 receptor with PD123319 impairs insulin signaling in C57BL/6 mice

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ABSTRACT

The renin-angiotensin system modulates insulin action. Angiotensin type 1 receptor exerts a deleterious effects while the angiotensin type 2 receptor (AT2R) appears to have beneficial effects providing protection against insulin resistance and type 2 diabetes. Although recent reports indicate that agonism of AT2R ameliorates diabetes and insulin resistance, the phenotype of AT2R-knockout mice seems to be controversial relating this aspect. Thus, in this study we have explored the role of AT2R in the control of insulin action. To that end, C57Bl/6 mice were administered the synthetic AT2R antagonist PD123319 for 21 days (10 mg/kg/day ip); vehicle treated animals were used as control. Glucose tolerance, metabolic parameters, in vivo insulin signaling in main insulin-target tissues as well as levels of adiponectin, TNF-α, MCP-1 and IL-6 in adipose tissue were assessed. AT2R blockade with PD123319 induced a marginal effect on glucose homeostasis but an important reduction in the insulin-induced phosphorylation of the insulin receptor and Akt in both liver and adipose tissue. Insulin signaling in skeletal muscle remained unaltered after treatment with PD123319, which could explain the minimal effect on glucose homeostasis induced by PD123319. Our current results reinforce the notion that the AT2R has a physiological role in the conservation of insulin action.

1. Introduction

Angiotensin (Ang) II is the main effector peptide of the renin-angiotensin system [1,2]. Ang II binds two distinct receptors, the angiotensin type-1 receptor (AT1R) and the angiotensin type-2 (AT2R) receptor, with high affinity [1,2]. The AT1R mediates most of the physiological actions of Ang II [1–3]. In contrast, AT2Rs opposes the actions of AT1Rs under the majority of circumstances [1–6]. Angiotensin type-2 receptors are expressed ubiquitously at very high levels in the fetus, but decline quickly in the neonatal period in most tissues [3,7]. Although there is a relatively low expression of AT2Rs compared to AT1Rs in adult tissues, AT2Rs are expressed in the adult kidney, adrenal cortex, heart and vasculature, and predominate over AT1Rs in specific sites such as the uterus, ovary, adrenal medulla, pancreas and discrete areas of the brain [7–11]. The AT2R, while being only barely expressed in most healthy tissues, is strongly upregulated following tissue damage [12]. The modulation of the counterbalance between AT1R and AT2Rs is the focus of intensive research given its potential therapeutic application in pathophysiological areas such as inflammation and insulin resistance [13]. Chronic Ang II elevation induces insulin resistance through activation of the AT1R [13]. Thus, AT1R blockers (ARBs) are known to improve insulin resistance and reduce the new onset of diabetes [13–15]. AT2R stimulation antagonizes the signals activated by AT1R in various tissues, improving insulin sensitivity and thus attenuating metabolic disorders [16]. The beneficial effects of AT2R stimulation has been established in various animal models of metabolic disorders [11,14,16–20]. The recent availability of the selective AT2R agonist, compound 21 (C21), has been a major breakthrough in this research area. In type 2 diabetic KKAy mice, C21 improved insulin sensitivity, increased adiponectin and reduced TNF-α levels, while protecting the pancreatic ß-cells [16]. In high-fructose/high-fat fed rats, C21 improved insulin sensitivity and glucose tolerance, while lowered triacylglyceride levels (TG) as well, an effect not seen by ARB treatment [17]. Similar results were obtained in high-fat diet fed mice, where C21 improved insulin sensitivity, reduced TNF-α, increased adiponectin and IL-10 levels, and reduced serum TG levels [18]. Notably, the effects of both C21 and valsartan seemed to be mediated via the AT2R as they were not observed in AT2R knockout (KO) mice [14]. Stimulation of pancreatic AT2R significantly improved insulin synthesis and secretion in

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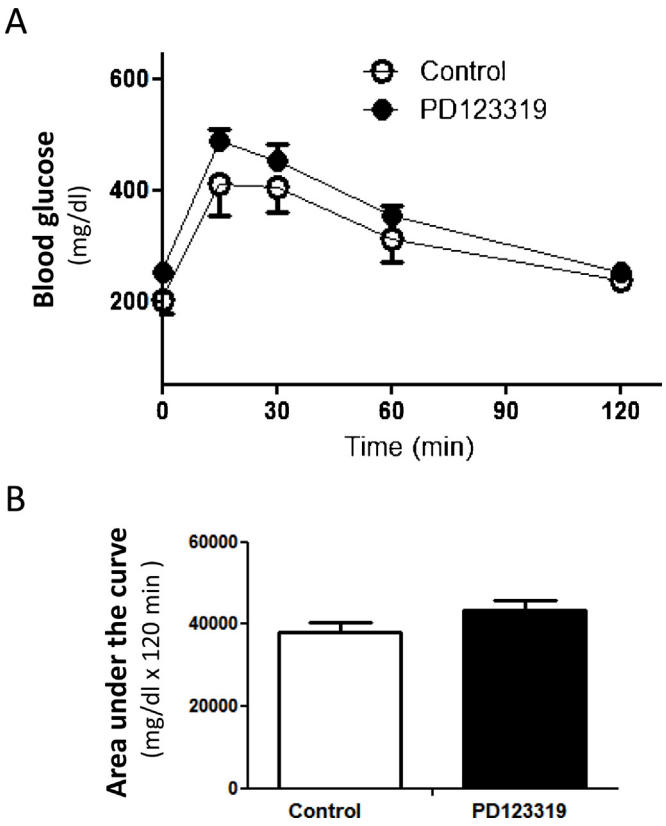

Fig. 1. Effects of PD123319 on glucose tolerance. Glucose tolerance test (A). Mice were fasted for 6 h and given an intraperitoneal injection of glucose (2 g/kg body wt). Data are presented as mean of plasma glucose levels (mg/dl) ± SEM from 14 mice in the control group and 14 in the PD123319-treated group. The bar graphs show the area under the curve during the glucose tolerance test (B).

adult rats [11]. Moreover, treatment with C21 prevented cell death of pancreatic ß-cell in diabetic rats [20].

In addition, pharmacological antagonism of the AT2R has been used to address this issue. The nonpeptide antagonist PD123319 (ditrifluoroacetate) is a widely used tool that has high affinity for the AT2 receptor (Ki ~ 10 nM) and is approximately 10,000-fold more selective for AT2 than AT1 receptors [21]. Acute infusion of PD123319 has been shown to decrease skeletal muscle glucose uptake in rats [22,23]. Systemic AT2 receptor blockade during the second transition in pancreatic development reduced the β-cell to α-cell ratio of the neonate islets, impaired their insulin secretory function and the glucose tolerance of the pups [24]. Unlike results obtained from AT2R stimulation or antagonism showing a participation of this receptor in insulin sensitivity and glucose homeostasis, information obtained from AT2R-KO mice has not been consistent so far [14,25–27]. Thus, the aim of the current work was to determine if the AT2R has a physiological importance in the preservation of insulin signaling and in the control of glucose homeostasis in normal mice. To that end C57BL/6 mice were administered during 3 weeks with PD123319. Metabolic parameters, glucose tolerance and the status of insulin signaling in main insulin target tissues was analyzed. Specifically we measured the phosphorylation levels of Tyr residues of the insulin receptor (IR) required for receptor tyrosine kinase activation (1158/1162/1163) and also Tyr 972 (required for docking of IR substrates and transmission of the signal downstream the IR) [28]. The in vivo phosphorylation status of the enzyme Akt, essential to the metabolic actions of insulin, was also analyzed. In particular phosphorylation at residues Thr308 and Ser473 required for full activation of this enzyme [28]. Additionally, we explored the phosphorylation status of the mitogen-activated protein kinase (MAPK) family, composed of extracellular signal-regulated kinase (ERK) 1/2, p38 MAPK and JNK. Both ERK 1/2 and JNK phosphorylate the IR and its cytosolic substrates IRS-1 and −2 at inhibitory Ser residues mainly in the liver, while p38 MAPK decreases expression of genes involved in insulin signaling, including GLUT-4 in both adipose tissue and skeletal muscle [28,29].

2. Materials and methods

2.1. Ethics

Ethics approval was granted by the Animal Ethics Committee at the School of Pharmacy and Biochemistry, University of Buenos Aires (Approval 57283/2015). This study was performed in accordance with all appropriate institutional and international guidelines and regulations for animal research.

2.2. Materials and reagents

The anti-insulin receptor (IR) β subunit (C19; sc-711), the rabbit polyclonal antibody that detects p38 MAPK when phosphorylated at Tyr182 (p-p38; sc-101759), the goat polyclonal antibody anti MCP-1 (R-17; sc-1785), the rabbit polyclonal anti IL-6 antibody (sc-1265-R), goat polyclonal anti-rabbit IgG conjugated with HRP (sc-2004), goat anti-mouse IgG-HRP (sc-2005) and rabbit anti-goat IgG-HRP (sc-2768) antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The polyclonal antibodies anti-phospho-IR-Tyr972 (07-838) and anti-phospho-IR/IGF1R-Tyr1158 (07-841) were from Millipore Corporation (Temecula, CA, USA). The rabbit polyclonal antibody anti-phospho-Akt-Ser473 (4060), the rabbit polyclonal antibody anti-phospho-Akt-Thr308 (9275), the anti-Akt (pan) rabbit monoclonal antibody (C67E7) that detects endogenous levels