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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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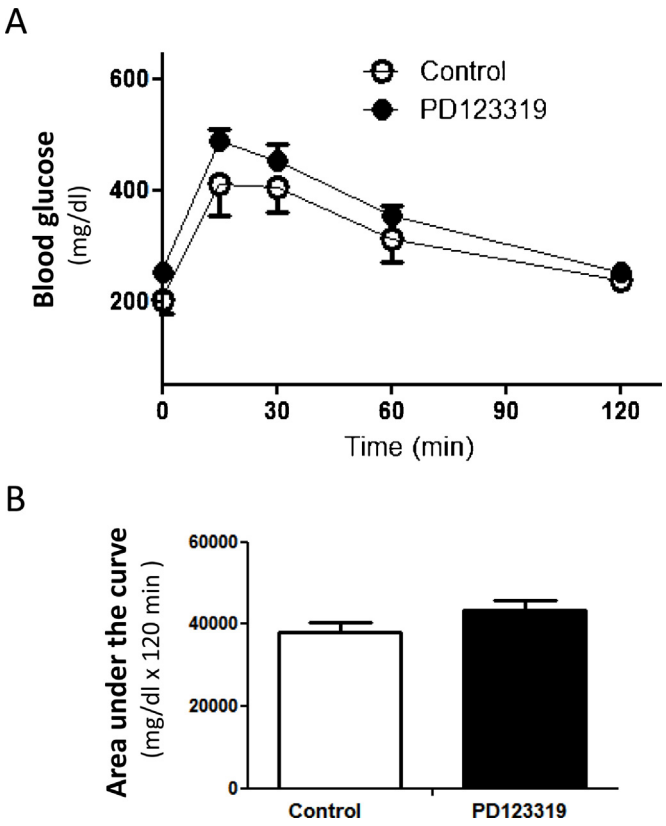

Fig. 1. Effects of PD123319 on glucose tolerance. Glucose tolerance test (A). Mice were fasted for 6 h and given an intraperitoneal injection of glucose (2 g/kg body wt). Data are presented as mean of plasma glucose levels (mg/dl) ± SEM from 14 mice in the control group and 14 in the PD123319-treated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

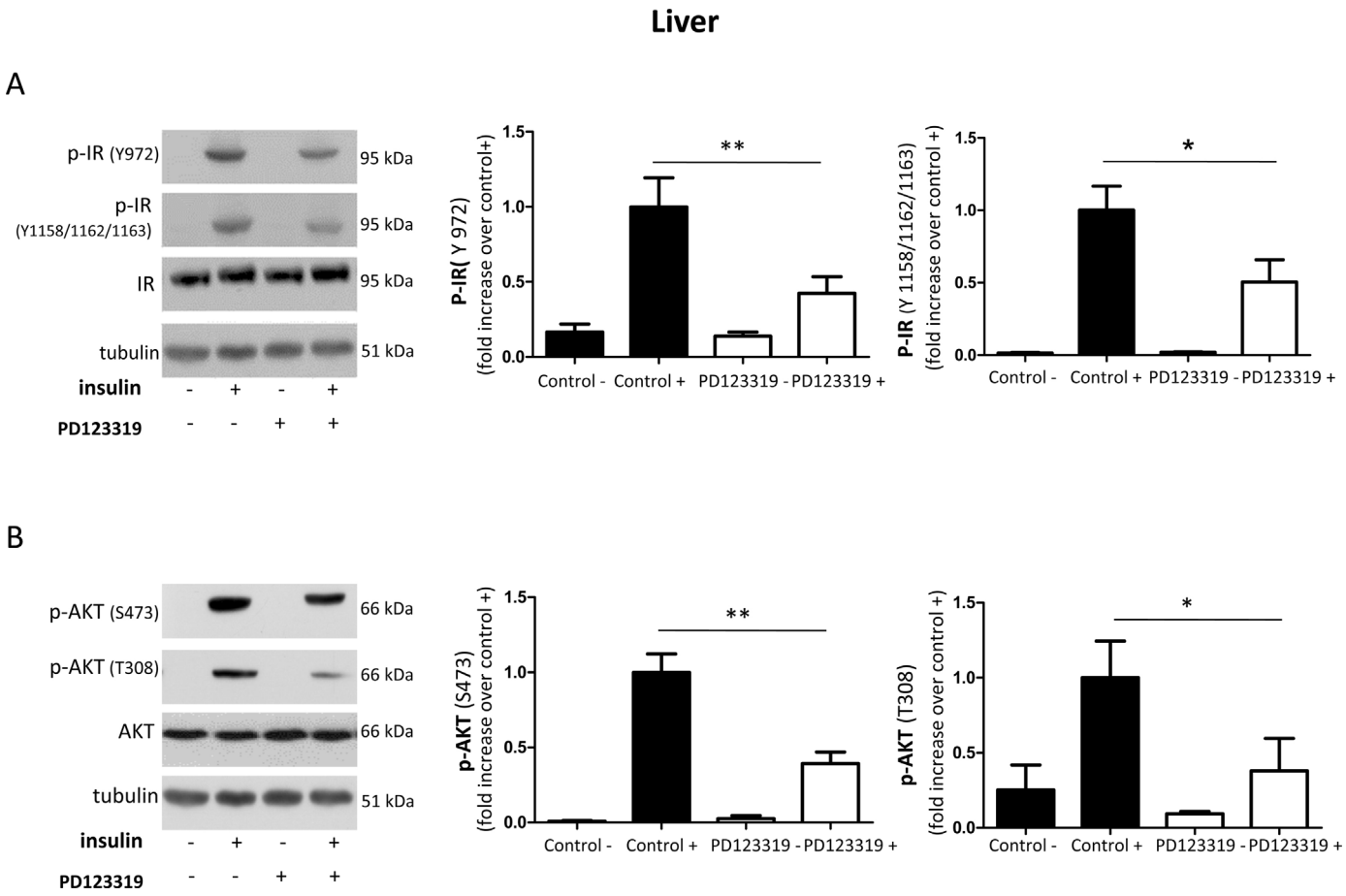

Fig. 2. Effects of treatment with PD123319 on the phosphorylation levels of the insulin receptor (IR); (A) and Akt (B) in liver of mice in basal conditions (−) and after acute in vivo insulin stimulation (+). Specific antibodies were used to detect phosphorylation of the IR at Tyrosine (Y) 972 and at the Tyrosine cluster (1158/1162/1163) and phosphorylation of Akt at Serine (S) 473 and Threonine (T) 308. $^{*}P < 0.05$, $^{**}P < 0.01$ vs insulin-stimulated control animals; n = 6 for all experiments. The observed molecular weight of each specific band is indicated to the right.

of total AKT1, AKT2, and AKT3 protein, the rabbit monoclonal antibody anti-JNK (9528), the rabbit monoclonal antibody anti-phospho-ERK1/2, that detects ERK1 and ERK2 (p44 and p42 MAPK respectively) when phosphorylated at Thr202 and Tyr204 (4370) and the rabbit polyclonal antibody anti-ERK1/2 (9102) were from Cell Signalling (Danvers, MA, USA). The goat polyclonal anti-TNF-α (AF-410) and anti-adiponectin (AF1119) antibodies were from R&D systems (Minneapolis, MN, USA). Protein loading in gels was evaluated with a polyclonal anti-β tubulin antibody (ab6046) from Abcam (San Francisco, USA).

2.3. Study design

Twelve week old male C57BL/6 mice were obtained from School of Veterinary Sciences, Universidad Nacional de La Plata (La Plata, Argentina). The animals were maintained under controlled light and temperature conditions, and had free access to water and standard chow diet. The total number of animals used was 28. To evaluate the effects of AT2R blockade on glucose metabolism and insulin sensitivity, animals were divided into two groups: animals treated with saline (control group); (n = 14), animals treated with PD123319 (PD123319 group); (n = 14). For 21 days, all animals received a daily intraperitoneal (i.p.) volume (0.1 ml) of saline solution (control) or PD123319 (10 mg/kg; SIGMA-Aldrich, St. Louis, MO, USA). Both the dose and administration interval of PD123319 were selected on the basis of previously published protocols [30–32].

2.4. Intraperitoneal glucose tolerance test (IPGTT)

We performed an IPGTT on day 18. Mice were fasted for 6 h (8:00 AM-14:00 AM) before the i.p.GTT. After the collection of an unchallenged sample (time 0), 2 g of glucose/kg body weight was administered into the peritoneal cavity. During the test, blood was collected from the tail vein 15, 30, 60, 90, and 120 min after a glucose load. Blood glucose levels were measured using an Accu-Check glucometer (Roche Diagnostics Corp., Indianapolis, IN, USA). All experimental protocols were performed in accordance with the international guidelines for animal care.

2.5. Acute insulin stimulation and tissue collection

At day 21, mice were fasted for 6 h (8:00 AM-14:00 AM) and then were anesthetized by the i.p. administration of a mixture of ketamine and xylazine (50 and 1 mg/kg, respectively), and submitted to the surgical procedure as soon as anesthesia was assured by the loss of pedal and corneal reflexes. The abdominal cavity was opened, the cava vein was exposed, and a dose of 10 IU porcine insulin/kg body weight in normal saline (0.9% NaCl), in a final volume of 0.2 ml, was injected via this vein. To obtain data under basal conditions, mice received an injection of diluent. The liver, adipose tissue (epididymal), and skeletal muscle (soleus), were removed after 1, 3, and 6 min, respectively. Serum was obtained from blood of saline- injected animals by centrifugation (3200*g* for 10 min at 4 °C). Tissues and a aliquot of serum (for insulin determination) were

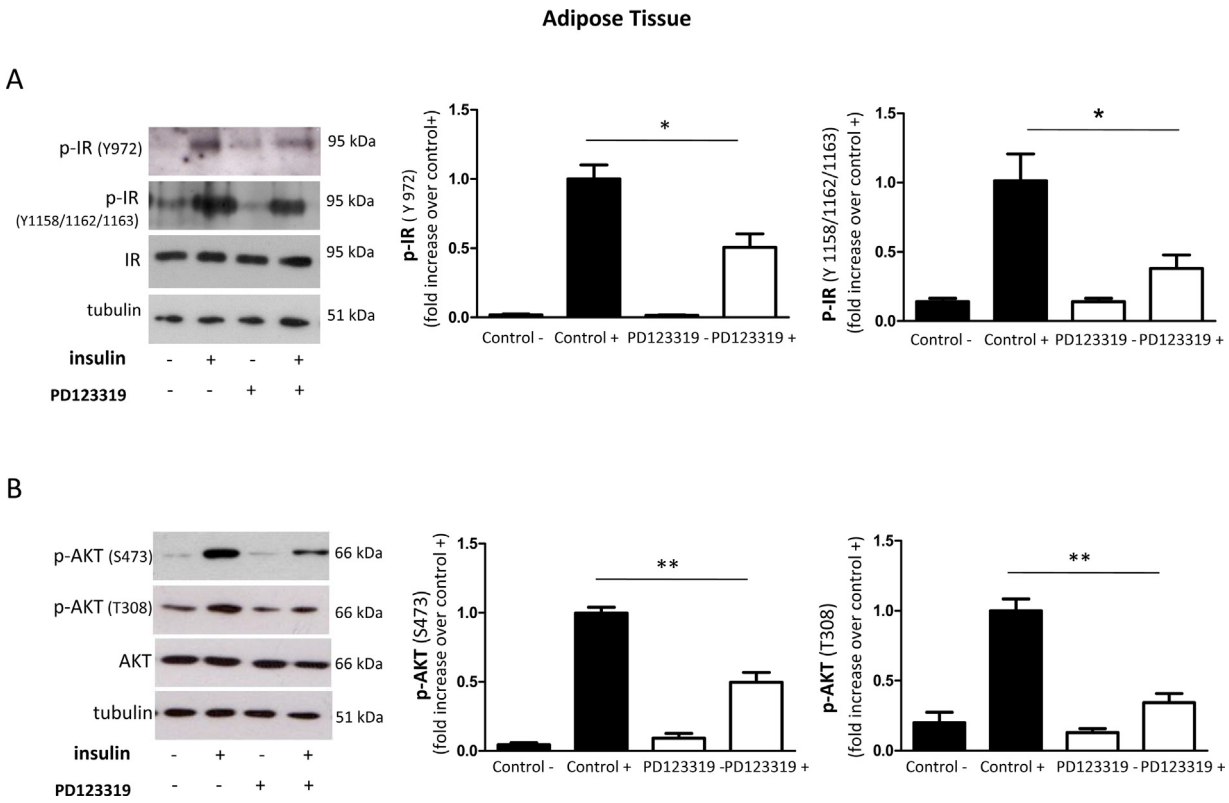

Fig. 3. Effects of treatment with PD123319 on the phosphorylation levels of the IR (A) and Akt (B) in adipose tissue of mice in basal conditions (−) and after insulin stimulation (+). Specific antibodies were used to detect phosphorylation of the IR at Tyrosine (Y) 972 and at the Tyrosine cluster (1158/1162/1163); phosphorylation of Akt at Serine (S) 473 and Threonine (T) 308.
*$P < 0.05$, **$P < 0.01$ vs insulin-stimulated control animals; n = 6 for all experiments.

kept at −80 °C until analysis. Another aliquot of serum was used for determination of circulating triglyceride (TG) and cholesterol concentrations trygliceridemia the same day of the sacrifice.

2.6. Tissue homogenization and western blotting

Tissue samples were homogenized in solubilization buffer containing 1% Triton together with phosphatase and protease inhibitors, as described previously [33,34]. Tissue extracts were centrifuged at 100,000*g* for 1 h at 4 °C to eliminate insoluble material, and protein concentration in the supernatants was measured using the bicinchoninic acid assay (Pierce BCA Protein Assay Reagent; Thermo Scientific,Waltham, MA, USA). Immunoblotting was performed according to previously described protocols [34]. Briefly, equal amounts of solubilized proteins (40 μg) were denatured by being boiled in reducing sample buffer and resolved by SDS-PAGE. Proteins were then transferred to PVDF membranes and immunoblotted with anti-phospho-IR, anti-phospho-Akt, anti-phospho-p38 MAPK, anti-phospho-JNK, and anti-phospho-Erk1/2 antibodies (1:2000 dillution for all antibodies). Protein abundance of IR, Akt, p38 MAPK, JNK, and Erk1/2 was detected in tissue lysates using the corresponding anti-protein antibodies at 1:2000 dillution. The abundance of IL-6, MCP-1, adiponectin and TNF-α in adipose tissue was detected in tissue lysates using specific antibodies at 1:2000 dillution; β tubulin was used as a loading control at a 1:10,000 dillution. After extensive washing, membranes were incubated with the appropriate secondary HRP-coupled antibody at 1:20,000 and processed for enhanced chemiluminescence (ECL) using the Pierce ECL plus Western Blotting detection system (ThermoFisher Scientific, Waltham, MA, USA). Bands were quantified using Gel-Pro Analyzer 4.0 (Media Cybernetics, Bethesda, MD, USA).

2.7. Glucose, insulin, triglycerides and cholesterol measurements

Blood glucose measurements were performed using a hand-held glucometer (ACCU-CHEK® Nano meter, Roche Diagnostics Corp., Indianapolis, IN). Circulating triglyceride (TG) and cholesterol concentrations were measured by an enzymatic colorimetric assay kit (Wiener Lab, Rosario, Argentina). Serum insulin levels were assessed using a mouse insulin ELISA kit (Ultra Sensitive Mouse Insulin ELISA Kit; Crystal Chem, Inc., Downers Grove, IL).

2.8. Statistical analysis

The data were analyzed using GraphPad Prism 5 (GraphPad software, San Diego, CA). All values are reported as means ± SEM unless specified otherwise. The statistical significance of differences in mean values between control and PD123319-treated animals was evaluated by Student's *t*-test. For insulin signaling analysis, differences in mean values between the four animal groups were assessed by two-way ANOVA followed by the Newman-Keuls multiple comparison test. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of PD123319 on glucose tolerance and metabolic parameters

Basal blood glucose level after 6 h of fasting did not differ between groups (Table 1). The response to an IPGTT at the termination of the 3-wk treatment with PD123319 is shown in Fig. 1. Plasma glucose levels increased significantly during the IPGTT in all groups of animals. However, the glucose tolerance curve peak and area

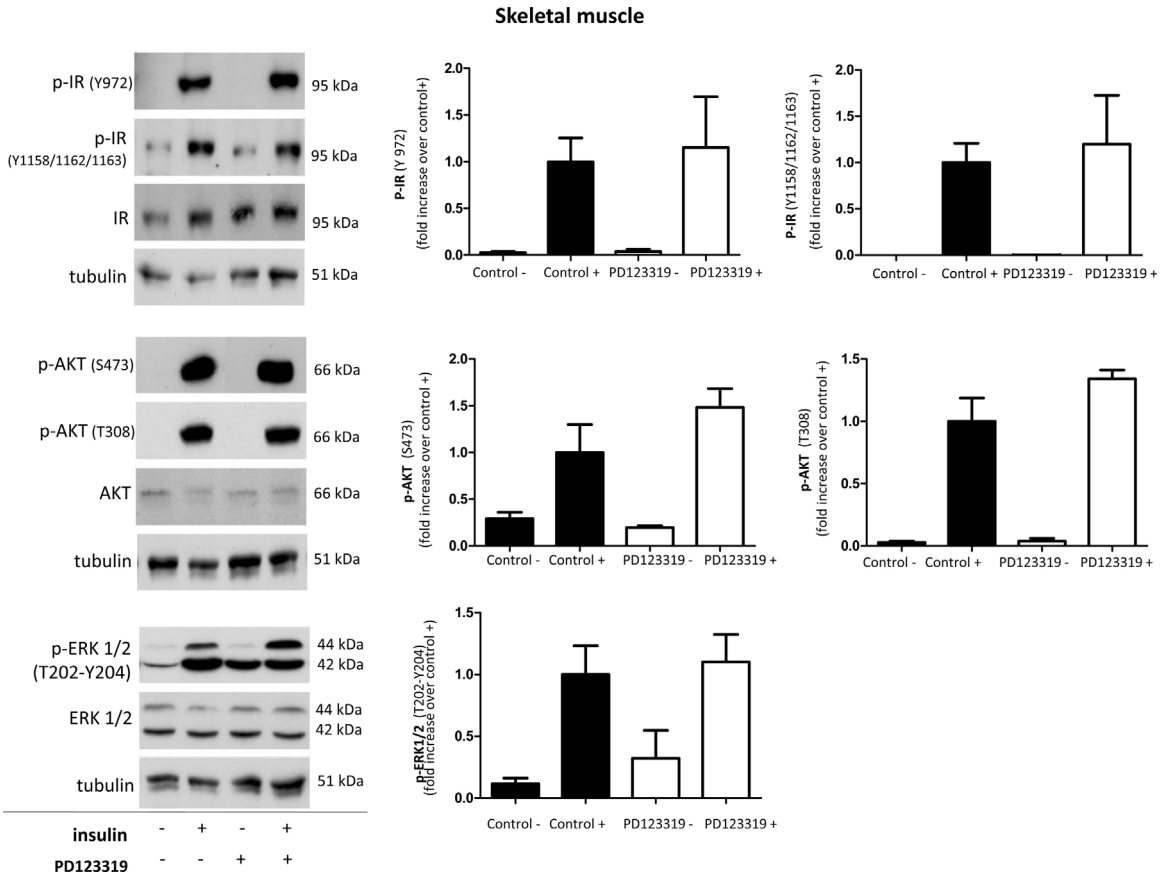

Fig. 4. Phosphorylation levels of the insulin receptor, Akt and ERK1/2 in skeletal muscle of mice chronically treated with PD123319 after acute administration of insulin (+) or vehicle (−). Specific antibodies were used to detect phosphorylation of the IR at Tyrosine (Y) 972 and at the Tyrosine cluster (1158/1162/1163); phosphorylation of Akt at Serine (S) 473 and Threonine (T) 308, and phosphorylation of ERK1/2 at activating residues Threonine (T) 202 and Tyrosine (Y) 204. n = 4 for all experiments.

Table 1
Body weight and metabolic parameters of the experimental animals.

Parameter	Control	n	PD123319	n
Body weight (g)	25 ± 0.5	(14)	24 ± 0.5	(14)
Glucose (mg/dL)	190 ± 8	(14)	199 ± 12	(14)
Insulin (ng/mL)	0.4 ± 0.1	(7)	0.7 ± 0.2	(7)
Triglycerides (mg/dL)	129 ± 8	(7)	135 ± 7	(7)
Cholesterol (mg/dL)	131 ± 8	(7)	132 ± 12	(7)

Values are means ± SEM. The number of samples used for each determination (n) is shown in parentheses.

under the curve (AUC) obtained in mice treated with PD123319 for 3 weeks (curve peak 490 ± 20 mg/dl; AUC 42292 ± 1892) was not statistically significant compared to control animals (curve peak 411 ± 55 mg/dl; AUC 38051 ± 2726); (P = 0.1 for the comparison of the AUC in PD123319-treated animals vs. control animals); (Fig. 1). Blood insulin concentrations were measured at baseline and remained unaltered after treatment with PD123319 (Table 1). No differences were observed in serum triglycerides and cholesterol levels between the control and PD123319-treated groups of animals (Table 1).

3.2. Effect of PD123319 on insulin signaling

Treatment with PD123319 for 3 weeks induced a significant impairment in insulin signaling in both liver and adipose tissue of male C57BL/6 mice (Figs. 2 and 3). In liver of PD123319-treated animals, insulin-induced phosphorylation levels of the insulin receptor (IR) at Tyr residues 1158/1162/1163 and 972 were reduced to 50% ($P < 0.01$) and 40%, respectively ($P < 0.05$), compared to values detected in insulin-stimulated control animals (Fig. 2A). This attenuation was also evident in adipose tissue (Fig. 3A). When compared to insulin-stimulated control animals, insulin-induced phosphorylation levels of the IR at Tyr1558/1162/1163 and Tyr972 were reduced in adipose tissue of PD123319-treated animals to 38% and 50% ($P < 0.05$); Fig. 3A).

In liver of PD123319-treated animals, insulin-induced phosphorylation levels of Akt were significantly reduced to 40% at Ser473 ($P < 0.01$) and to 38% at Thr308 ($P < 0.05$), when compared to values obtained in insulin-stimulated control mice (Fig. 2B). In adipose tissue of mice treated with PD123319, insulin-induced phosphorylation of Akt was also greatly reduced when compared to insulin-stimulated control mice [50% (Ser473), and 40% (Thr308); $P < 0.01$ in both cases; Fig. 3A]. These findings represent a solid confirmation that AT2R blockade induces an impairment of the signaling branch responsible for the metabolic actions of insulin in liver and adipose tissue. Insulin-stimulated levels of IR and Akt phosphorylation in skeletal muscle were not affected by PD123319 administration (Fig. 4), indicative of unaltered insulin sensitivity in this tissue after AT2R blockade.

3.3. Investigation of potential mechanisms of reduced insulin sensitivity associated with treatment with PD123319

As shown in Fig. 5A, the reduction in the response to insulin observed in liver of PD123319-treated animals, correlated with a marked increase (58%) in the activation of ERK1/2 in this tissue ($P < 0.05$), while no statistically significant changes were observed in the levels of phosphorylation of JNK and p38 MAPK at residues required for kinase activation (Fig. 5B and C respectively). In adipose

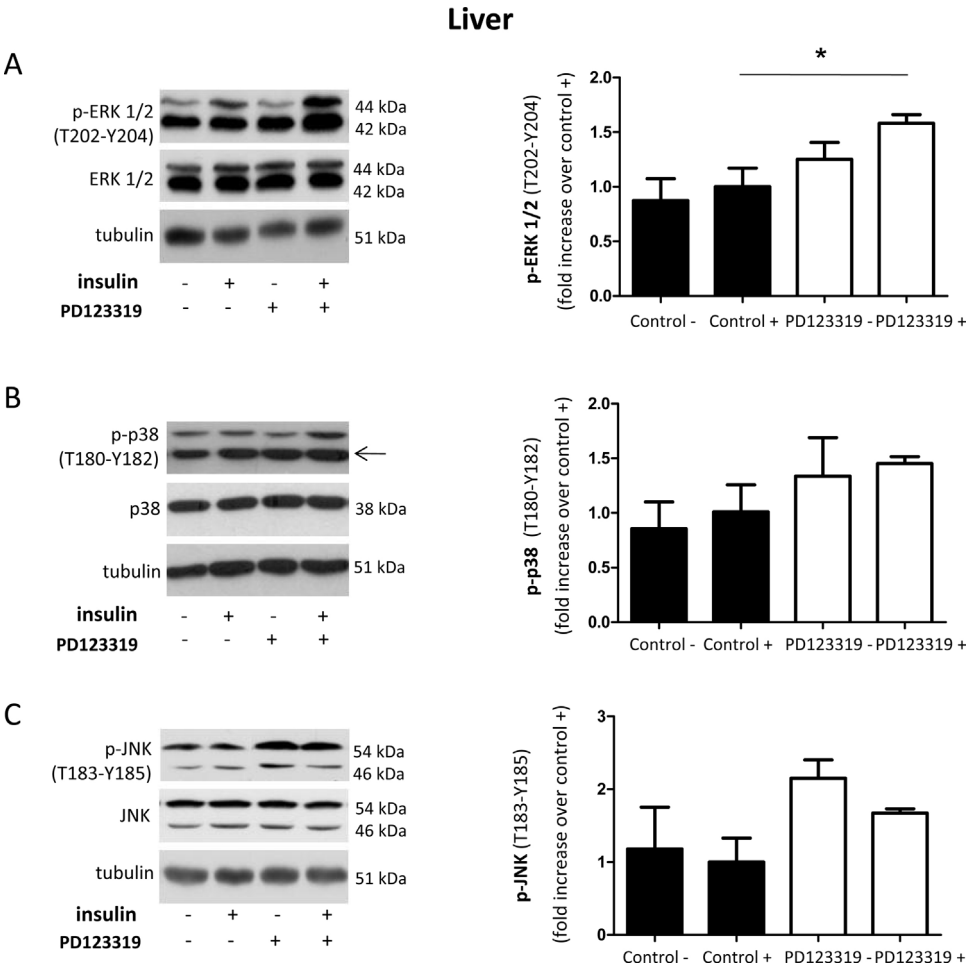

Fig. 5. Effects of treatment with PD123319 on phosphorylation/activation status of ERK1/2 (A), p38 MAPK (B), and JNK (C) in liver. Specific antibodies were used to detect phosphorylation at residues indicative of enzyme activity. For ERK1/2, Threonine (T) 202 and Tyrosine (Y) 204; for p38 Threonine 180 (T) and Tyrosine (Y) 182, and for JNK Threonine (T) 183 and Tyrosine (Y) 185. n = 6 for all experiments.

tissue of PD123319-treated mice, although there was a tendency towards increased ERK and JNK phosphorylation, these changes were not statistically significant (Fig. 6A and C). Phosphorylation levels of p38 in adipose tissue were unaltered after treatment with PD123319 (Fig. 6B).

3.4. *Effect of PD123319 on tissue abundance of adiponectin and inflammatory cytokines in adipose tissue*

Insulin signaling in adipose tissue was greatly affected after AT2 blockade. To investigate the mechanism behind this alteration, the levels of the adipocytokine adiponectin and of the inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) were determined. As shown in Fig. 7, MCP-1 levels in adipose tissue remained unaltered after AT2R blockade. The same pattern was obtained for the levels of adiponectin, IL-6 and TNF-α in adipose tissue (Fig. 7).

4. Discussion

Recent findings point towards a participation of the AT2R in the modulation of insulin action. Pharmacological stimulation of the AT2R improves insulin action in various animal models of insulin resistance [16–20]. However, the metabolic phenotyping of AT2RKO mice have led to discrepancies. Experiments performed by Shiuchi et al. demonstrated that AT2R deficiency in mice abrogated insulin-mediated glucose uptake in white adipose tissue while preserving skeletal muscle glucose uptake [14]. In contrast, Yvan-Charvet et al. reported an improvement of glucose tolerance and insulin sensitivity in AT2RKO mice [25]. Samuel et al. reported normal glucose tolerance with a tendency to an impairment in AT2R-deficient mice [26]. Recently, Noll et al. reported that compared to the wild-type mice, AT2R-deficient mice had an increase in fasting blood glucose and a decrease in plasma insulin and leptin levels [27]. To further clarify the participation of the AT2R in the modulation of insulin action, in the current study we analyzed the effects of chronic AT2R blockade with PD123319 on glucose tolerance, circulating levels of insulin, triglycerides and cholesterol, as well as the status of the insulin signaling system in liver, adipose tissue and skeletal muscle. We found that in C57BL/6 mice, chronic AT2R antagonism by administration of PD123319 induced an important reduction of the insulin-induced phosphorylation levels of the IR and Akt in both liver and adipose tissue. The impairment of insulin signaling induced by AT2R blockade seems tissue-specific since insulin signaling remained essentially unaltered in skeletal muscle of PD123319-treated animals. This deleterious effect of PD123319 on insulin signaling points to an important role of AT2R as a regulator of insulin action.

PD123319 is a potent, selective, non-peptide angiotensin AT2R antagonist [35]. Its acute metabolic effects have been previously studied in rats under acute systemic infusion for 120 min using a dose of 50 μg/kg per min [22,23]. Under this experimental setting, acute AT2R blockade,induced a rapid attenuation of

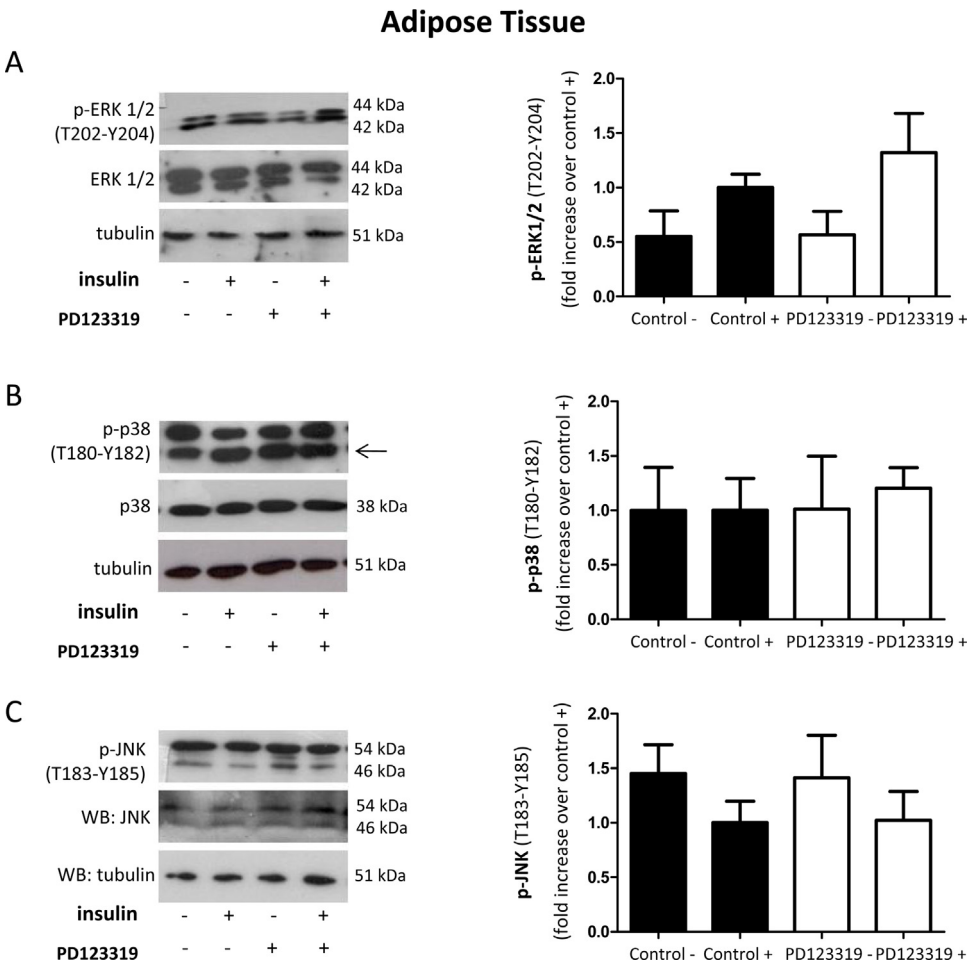

Fig. 6. Effects of treatment with PD123319 on phosphorylation/activation status of ERK1/2 (A), p38 MAPK (B), and JNK (C) in adipose tissue. n = 6 for all experiments.

insulin-stimulated whole-body glucose disposal, which was associated with abrogation of insulin-stimulated muscle microvascular recruitment, nitric oxide production and muscle Akt phosphorylation [22,23]. In the current study we found that PD123319 administered for 3 weeks at a dose of 10 mg/kg induced a considerable attenuation of insulin-stimulated IR/Akt in liver and adipose tissue while skeletal muscle remained unaffected. Discrepancies observed in the effects induced by PD123319 on insulin signaling in skeletal muscle could be attributed to differences in the experimental settings (dose, species, route of administration and duration of treatment). In spite of this, both our current findings and previous reports add to the notion that the AT2R has a physiological role in glucose handling and insulin action.

Although PD123319 was suggested to interact with the AT1R at high doses [36], recent studies based on ligand binding demonstrated that PD123319 lacks affinity for AT1R (IC50 values >10^{-6}M); [37]. Also, in vitro at high doses (10^{-6}–10^{-7} M), PD123319 has been reported to block Mas and MrgD, receptors for Ang-(1–7) and alamandine respectively [38,39]. At the dose used in our current study, and considering an affinity for the AT2R of 10 nM, PD123319 would be expected to interact only with AT2Rs. However, interaction with other receptors of the renin-angiotensin system cannot be ruled out.

Concerning the site of action of PD123319, in the adult rat, AT2R has been shown to be present in liver, adipose tissue, skeletal muscle although in low amounts in comparison to AT1R [11]. AT2R protein abundance is high in the pancreas, and it seems to have an insulinotropic effect [11]. This correlates well with recent reports showing reduced insulin levels in AT2RKO mice [27]. Accordingly, we expected that global blockade of AT2R by PD123319 administration could lead to hypoinsulinemia concomitant with hyperglycemia. However, despite the clear attenuating effects of PD123319 on insulin signaling in liver and adipose tissue, the observed effects in vivo on glucose homeostasis and plasma insulin concentrations remained marginal. This might be partly explained by the lack of modification of insulin signaling in skeletal muscle after AT2R blockade, given that this tissue is a major regulator of glucose homeostasis. Also, the experiments were performed in healthy animals with naturally well-balanced glucose homeostasis. The deleterious effects of PD123319 could probably become more obvious in models with increased Ang II levels, were exacerbation of the actions of AT1R will become evident.

Obesity is closely linked to a chronic inflammatory state, which contributes to metabolic disorders [40]. Adipose tissue is thought to directly exacerbate insulin resistance and to trigger inflammation by secreting adipokines such as IL-6 and TNF-α [29]. Thus, dysfunction of this tissue can tend towards a chronic inflammatory state through imbalance of pro-inflammatory and anti-inflammatory activities resulting in worsening of insulin resistance [41,42]. Also, TNF-α is known to directly inhibit insulin signaling, resulting in insulin resistance [43]. It has been reported that AT2R stimulation attenuates inflammation in white adipose tissue and thereby help enhance adipocyte differentiation and improve insulin resistance [16]. We found that PD123319 treatment did not modify the expression of TNF-α, IL-6 and MCP-1 in adipose tissue. This could be interpreted as AT2R having a protective role in terms of inflam-

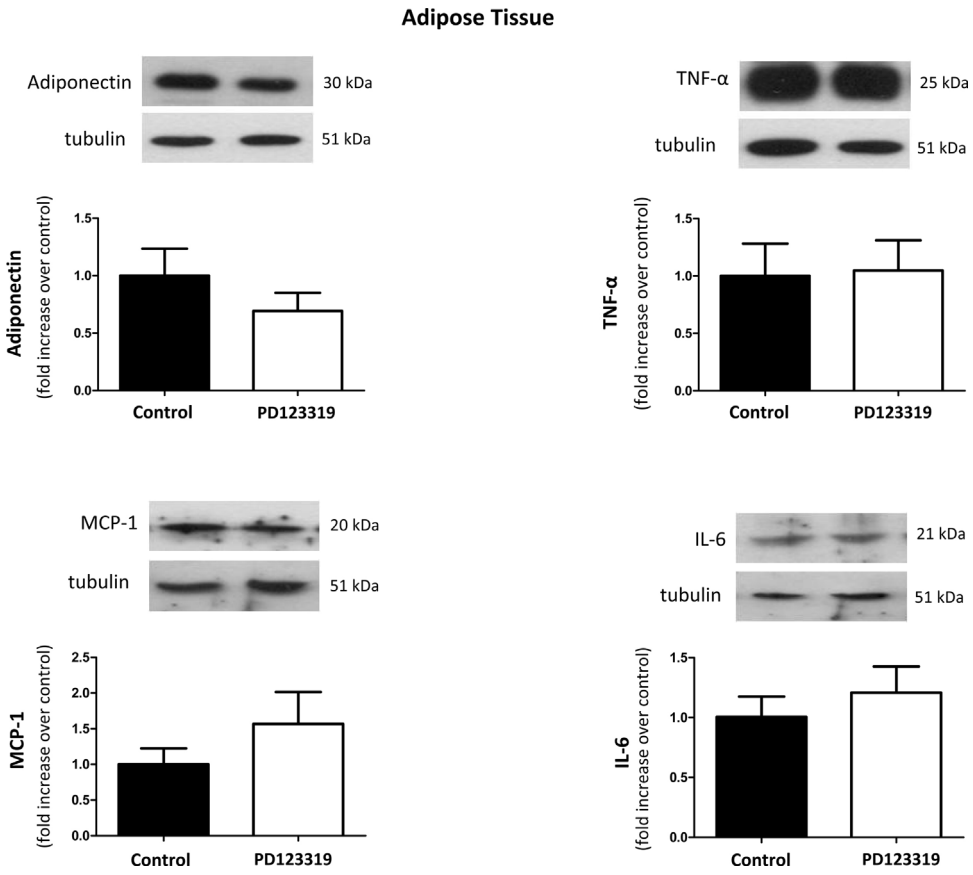

Fig. 7. Effects of treatment of mice with PD123319 on the levels of adiponectin, tumor necrosis alpha (TNF-α), interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) in adipose tissue. n = 12 for all determinations.

mation only under pathological conditions and that this would not apply under normal physiological conditions.

Taken together, current results demonstrate that AT2R activity seems to be required for normal insulin signaling in both liver and adipose tissue of C57Bl/6 mice. Particularly in the liver, this attenuation of insulin signaling correlated with increased levels of the Ser kinase ERK1/2. Present results add to the current notion that considers that pharmacologic manipulation aimed at increasing the AT2R-to-AT1R activity ratio may have the potential to improve insulin sensitivity and glucose metabolism and to reduce the cardiovascular complications associated with diabetes and insulin resistance.

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