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Translational

Thiazolidinediones attenuate lipolysis and ameliorate dexamethasone-induced insulin resistance

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ARTICLE INFO

Article history:
Received 5 December 2014
Accepted 18 February 2015

Keywords:
Thiazolidinedione
Lipolysis
Free fatty acids
Dexamethasone
Insulin resistance

ABSTRACT

Background. Elevated levels of circulating free fatty acids induce insulin resistance and often occur in obese and diabetic conditions. One pharmacological basis for the antidiabetic effects of thiazolidinediones (TZDs) is that TZDs reduce levels of circulating FFAs by accelerating their uptake and reesterification from plasma into adipocytes. Here, we investigated whether TZDs affect adipose lipolysis, a process controlling triglyceride hydrolysis and FFA efflux to the bloodstream.

Methods. The effects of TZDs on lipolysis were investigated in primary rat adipocytes *in vitro* and in rats *in vivo*.

Results. In rat primary adipocytes, the TZDs pioglitazone, rosiglitazone and troglitazone inhibited the lipolytic reaction dose- and time-dependently and in a post-receptor pathway by decreasing cAMP level and total lipase activity. TZDs increased the phosphorylation of Akt/protein kinase B, an action required for activating cyclic-nucleotide phosphodiesterase 3B, a major enzyme responsible for cAMP hydrolysis in adipocytes. Furthermore, rosiglitazone inhibited the lipolytic action in dexamethasone-stimulated adipocytes, thereby preventing the increased level of circulating FFAs, and ameliorated insulin resistance *in vivo* in dexamethasone-treated rats.

Conclusions. TZDs may attenuate lipolysis and FFA efflux by activating Akt signaling to decrease cAMP level and hence reduce lipase activity in adipocytes. Inhibiting lipolysis and FFA efflux with TZDs could be a pharmacological basis by which TZDs antagonize diabetes, particularly in patients with hypercortisolemia or glucocorticoid challenge.

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Abbreviations: TZDs, thiazolidinediones; FFAs, free fatty acids; TNF-a, tumor necrosis factor-α; PPARγ, peroxisome proliferator-activated receptor; ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase.

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http://dx.doi.org/10.1016/j.metabol.2015.02.005
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1. Introduction

Elevated plasma free fatty acids (FFAs) restrict glucose utilization through the Randle glucose–fatty acid cycle and induce insulin resistance [1]. The lipolytic reaction in adipose cells governs the breakdown of triglycerides and the release of glycerol and FFAs, which are transported in the plasma to supply various tissues. In general, adipocyte lipolysis is maintained at a basal level under quiescent conditions and is physiologically stimulated by catecholamines, which are elevating cellular cAMP production and activating cAMP-dependent protein kinase. Other hormones and cytokines, such as glucocorticoids [2] and tumor necrosis factor-α (TNF-α) [3–5], induce chronic lipolysis via different mechanisms. Obesity and type 2 diabetes mellitus are associated with elevated circulating levels of FFAs, which are thought to directly promote insulin resistance [1,6–8], perhaps because adipocytes in obese and diabetic subjects have a higher rate of basal lipolysis and thus higher levels of FFA release to the circulation [1,9].

Thiazolidinediones (TZDs) are selective agonists of peroxisome proliferator-activated receptor γ (PPARγ) and are used as insulin-sensitizing agents for treating diabetes. TZDs improved insulin sensitivity links to reduce circulating levels of FFAs [10,11]. TZDs activate the expression of numerous PPARγ-targeting genes to accelerate FFA uptake [12–14] and reesterification [15,16] in adipocytes. PPARγ ligand inhibits the lipolytic action of TNF-α [17,18], a cytokine with high production from adipose tissue of obese subjects. Thus, TZDs may decrease plasma FFA levels through multiple mechanisms.

In the present study, we investigated whether TZDs could attenuate basal lipolysis in primary adipocytes via phosphorylation of Akt/protein kinase B (PKB) to decrease intracellular cAMP production and lipase activity. Recently, we found that glucocorticoids directly stimulated lipolysis and increased FFA efflux from adipocytes to the bloodstream [2], and here we further revealed that TZDs blocked lipolysis and FFA efflux in dexamethasone-stimulated adipocytes *in vitro* and ameliorated insulin resistance in dexamethasone-challenged rats *in vivo*. Our results provide new insight into the multiple pharmacological bases by which TZD improves insulin resistance in diabetic patients, particularly those with hypercortisolemia or glucocorticoid challenge.

2. Methods

2.1. Materials

Troglitazone, pioglitazone and rosiglitazone were from Cayman Chemical; LY294002 was from Cell Signaling Technology (Beverly, MA). [^{3}H]-triolein was from PerkinElmer (Boston MA). Rabbit antibodies raised against perilipin-1 [19] or hormone-sensitive lipase (HSL) were gifts from C. Londos (US National Institutes of Health). Rabbit anti-Akt and anti-phospho-Akt (Ser 473) antibodies were from Santa Cruz Biotechnology. p-PKA substrate, p-PI3K p85, P85, P110α, p-HSL (Ser 565) and p-HSL (Ser 660) antibodies were from Cell Signaling Technology. Enhanced chemiluminescence detection reagents were from Applygen Technologies (Beijing, China).

2.2. Animals

Rat studies were performed under an approved protocol by the Institutional Animal Care and Use Committee at Sichuan University. Rats (150 ± 5 g) were randomly divided into 4 groups (n = 6 each) for treatment: (1) vehicle control; (2) rosiglitazone; (3) dexamethasone; and (4) dexamethasone plus rosiglitazone. Dexamethasone (0.1 mg/kg/day) in saline was injected subcutaneously at 9:00 a.m. for 6 weeks. Rosiglitazone (5 mg/kg/day) dissolved in 0.5% methylcellulose was administered by oral gavage over the same period. Control rats underwent vehicle injection (saline) and gavage (0.5% methylcellulose). The rats were fed *ad libitum*. At 5 weeks, rats were fasted for 12 h and were administered glucose (2.5 g/kg) for oral glucose tolerance test (OGTT). Plasma insulin was measured by ^{125}I-radioimmunoassay (Fu-Rui Biotechnologies, Beijing). Plasma levels of glucose, FFAs, glycerol, triglycerides and total cholesterol were determined by use of commercial assay kits (Applygen Technologies, Beijing).

2.3. Isolation and culture of primary rat adipocytes

Primary adipocytes were isolated from rat epididymal fat pads as we described previously [2,20]. The fat pads (~1 g) were minced and digested in Krebs–Ringer solution containing 0.75 mg/ml type I collagenase, 200 nM adenosine, 1% defatted BSA, and 25 mM HePes, pH 7.4. After incubation for 40 min at 37 °C in a water bath shaken at 100 cycles/min, cells were filtered through nylon mesh, washed 3 times in warmed DMEM, then packed by centrifuging at 200×g for 3 min to determine the packed cell volume (PCV) of adipocytes [20], the proportion of adipocytes in cell suspension. One milliliter of packed adipocytes was suspended in 9 ml DMEM containing 200 nM adenosine and 1% defatted BSA, with PCV 10%. Adipocytes were preincubated in an atmosphere of 5% CO_2 at 37 °C for 1 h before treatments.

2.4. Glycerol assay

The incubation medium was heated at 70 °C for 10 min to inactivate residue lipase activity in samples. Glycerol content in culture medium was an index of lipolysis and was determined at absorption at 490 nm [4,20] with use of a colorimetric assay kit from Applygen Technologies (Beijing). Lipolysis data were expressed as micromoles of glycerol or FFA per milliliter PCV of adipocytes.

2.5. FFA assay

The level of FFAs in culture medium and serum was determined by a colorimetric assay as we described previously [2,4].

2.6. Immunoblotting

After treatment, each 50 μl of packed adipocytes was lysed in sample buffer containing 62 mM Tris–HCl, pH 6.8, 5% SDS, 0.1 mM Na_3VO_4, and 50 mM NaF. The protein content was determined by the Lowry protein assay. Equal amounts of proteins were loaded and separated by SDS-PAGE. The proteins transferred on membranes were recognized with

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