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Effect of thiazolidinediones on equilibrative nucleoside transporter-1 in human aortic smooth muscle cells

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Abstract

Thiazolidinediones are a new class of anti-diabetic agents which increase insulin sensitivity by binding to the peroxisome proliferator-activated receptor γ (PPARγ) and stimulating the expression of insulin-responsive genes involved in glucose and lipid metabolism. These drugs also have vasodilatory and anti-proliferative effects on vascular smooth muscle cells. However the mechanisms for these actions are not fully understood. Adenosine is a vasodilator and a substrate of equilibrative nucleoside transporters (ENT). The present study studied the effects of three thiazolidinediones, troglitazone, pioglitazone and ciglitazone, on ENT1 in the human aortic smooth muscle cells (HASMCs). Although incubating HASMCs for 48 h with thiazolidinediones had no effect on ENT1 mRNA and protein levels, troglitazone acutely inhibited [^{3}H]adenosine uptake and [^{3}H]NBMPR binding of HASMCs with IC_{50} values of 2.35 ± 0.35 and 3.99 ± 0.57 μM, respectively. The effect of troglitazone on ENT1 was PPARγ-independent and kinetic studies revealed that troglitazone was a competitive inhibitor of ENT1. In contrast, pioglitazone and ciglitazone had minimal effects on [^{3}H]adenosine uptake by HASMCs. Troglitazone differs from pioglitazone and ciglitazone in that its side-chain contains a Vitamin E moiety. The difference in structure of troglitazone did not account for its inhibitory effect on ENT1 because Vitamin E did not inhibit [^{3}H]adenosine uptake by HASMCs. Using the nucleoside transporter deficient PK15NTD cells stably expressing ENT1 and ENT2, it was found that troglitazone inhibited ENT1 but had no effect on ENT2. From these results, it is suggested that troglitazone may enhance the vasodilatory effect of adenosine by inhibiting ENT1. Pharmacologically, troglitazone is a novel inhibitor of ENT1.

Keywords: Thiazolidinediones; Troglitazone; Adenosine; Diabetes; Nucleoside transporter; Smooth muscle cells

1. Introduction

Thiazolidinediones are a group of structurally related compounds with a common thiazolidine-2,4-dione ring attached to different side-chains. These compounds are used for the treatment of type 2 diabetics. Thiazolidinediones increase insulin sensitivity in a peroxisome proliferator-activated receptors gamma (PPARγ)-dependent manner by promoting the expression of insulin responsive genes in order to reduce blood glucose levels and correct hyperinsulinemia [1–4]. Conversely, PPARγ-independent actions of thiazolidinediones have also been reported, including direct dilatory and anti-proliferative effects on vascular smooth muscle cells (SMCs) [5–10] as well as rapid inhibition of L-type calcium channels [7,9,10].

Adenosine is the most important physiological nucleoside. It acts through G-protein coupled receptors to exert diverse effects on cellular functions [11]. It is released in response to cell injury and stress to modulate cell and organ energy demand and consumption. For instance, hypoxia and ischemia lead to an increase in extracellular adenosine which causes vasodilation by acting through A_2 adenosine receptors on vascular SMCs [12] and thus increases blood flow and oxygenation. Interestingly, adenosine has also been shown to inhibit growth of aortic SMCs [13]. Since adenosine is a substrate of nucleoside transporters, nucleoside transporters play integral roles in adenosine functions by regulating adenosine levels in the vicinity of adenosine

Abbreviations: ENT, equilibrative nucleoside transporter; HASMCs, human aortic smooth muscle cells; NBMPR, nitrobenzylmercaptopurine riboside; PPARγ, peroxisome proliferator-activated receptor-gamma

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receptors [14]. Recently, it was shown that diabetes changes ENT1 message and/or protein expression. ENT1 message expression in heart, liver and kidney of streptozotocin-induced diabetic rats are decreased [15]. Interestingly, diabetes induces opposite changes in ENT1 activity and message in vascular endothelial cells and SMCs [16,17]. We previously showed that HASMCs contain ENT1 as their sole functional nucleoside transporter and glucose up-regulates ENT1 activity, protein and message levels [18]. These results suggested that the increase in ENT1 activity in diabetes may affect the availability of adenosine in the vicinity of adenosine receptors and thus alter vascular functions in diabetes. Since thiazolidinediones activate PPARγ to regulate expression of genes at transcriptional levels, in the present study, we determined whether thiazolidinediones would prevent the glucose-induced stimulation of ENT1 activity, protein and message levels in HASMCs. This might provide a possible mechanism for alternation of vascular functions in diabetes by thiazolidinediones as the availability of adenosine to its receptors might be changed.

2. Materials and methods

2.1. Culture of HASMCs

HASMCs were obtained from American Tissue Culture Collection (Manassas, VA) and cultured in DMEM (containing 5 mM glucose) supplemented with 10% (v/v) fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in 95% air–5% CO_2. Forty-eight hours prior to uptake study and mRNA and protein isolation, cells were incubated in serum-free DMEM and were grown in the presence of either 5 mM (control) or 25 mM glucose and with and without thiazolidinediones as described in each figure legend.

2.2. Adenosine uptake

All experiments were carried out in Na^+-free buffer containing (in mM): 140 *N*-methyl-D-glucamine (NMDG), 5 HEPES, 5 KH_2PO_4, 1 $CaCl_2$, 1 $MgCl_2$ and 10 D-glucose (pH 7.4), because the nucleoside transport in HASMCs is sodium-independent. Confluent monolayers of cells in 24-well plates were washed three times in Na^+-free buffered solution. Three hundred microlitres of Na^+-free buffered solution containing [^{3}H]adenosine (10 μM, ±10 μM NBMPR, 2 μCi/ml) was then added to each well for 1 min. To study the effects of thiazolidinedione, drugs (30 μM) were added to the cells simultaneously with [^{3}H]adenosine. The plates were then washed three times rapidly with ice-cold PBS containing (in mM): 137 NaCl, 2.68 KCl, 1.47 KH_2PO_4 and 8.1 Na_2HPO_4 (pH 7.4). Cells were solubilized in 0.5 ml of 5% (v/v) Triton X-100. The radioactivity was measured by a β-scintillation counter. The protein content was determined spectrophotometrically using a commercial bicinchoninic acid assay (Pierce Biochemicals, Rockford, IL).

2.3. RNA isolation and RT-PCR

Total RNA was isolated from HASMCs using TRIzol reagent (Invitrogen, Grand Island, NY). Two micrograms of total RNA were used for first strand cDNA synthesis using random hexamer primers and superscript II RNase H^- reverse transcriptase (SuperScript Preamplification System, Invitrogen). The resulting first strand cDNA was directly used for PCR amplification.

The two primers for amplifying ENT1 (accession number NM_004955) were sense 5′-GACATGTCCCAGAATGTGTCC-3′ (corresponding to nucleotides 308-328) and antisense 5′-GGGTCCTTCAAGCTTGAGCTG-3′ (corresponding to nucleotides 887-907), which generated a 600-base pair (bp) PCR product. To semi-quantify the PCR products of ENT1, optical density values of nucleoside transporter bands were normalized to those of β-actin. The two primers for amplifying β-actin (accession number NM_001101) were sense 5′-GGCGTGATGGTGGGCATG-3′ (corresponding to nucleotides 197-214) and antisense 5′-CTGGGTCATCTTCTCGCG-3′ (corresponding to nucleotides 419-436), which yielded a PCR product of 240-bp. Reactions were carried out for 30 cycles with the following parameters: denaturation at 94 °C for 30 s, annealing at 55 °C for 1 min and extension at 72 °C for 1.5 min. PCR products were analyzed by agarose gel electrophoresis and visualized by staining with ethidium bromide.

2.4. Western blotting

Polyclonal anti-ENT1 antibody was raised in rabbit as previously described [19]. HASMCs were grown to confluence on 10-cm Petri dishes. All subsequent manipulations of the cells were conducted at 4 °C with ice-cold solutions. The cells were washed three times with PBS, scraped in 2 ml of 5 mM sodium phosphate, pH 8, with a protease inhibitor cocktail (Sigma, St. Louis, MO) (1:100, v/v). Cells were sonicated briefly and centrifuged at 3000 × *g* for 10 min to remove nuclei and unbroken cells. The resulting supernatant was centrifuged at 30,000 × *g* for 30 min to pellet the crude microsomal membranes, which was resuspended in 5 mM sodium phosphate. The crude membranes were then resolved on 9% (w/v) SDS-polyacrylamide gels and electrotransferred onto nitrocellulose membranes. After blocking with 5% (w/v) nonfat dry milk in PBS overnight at 4 °C, nitrocellulose membranes were incubated with the anti-hENT1 antibody (1:100, v/v, dilution in blocking solution) at room temperature for 2 h. Nitrocellulose membranes were then washed extensively with 0.02% (v/v) Triton X-100 in PBS. After washing, the membranes were incubated with horseradish-conjugated goat anti-rabbit secondary anti-

body (1:5000, v/v, dilution in blocking solution) at room temperature for 2 h. Excess secondary antibody was again washed, and the bound secondary antibody was detected by enhanced chemiluminescence (Western Blot Chemiluminescence Reagent Plus; NEN Life Science Products, Boston, MA). Protein expression of β-actin was similarly detected with the monoclonal mouse anti-actin antibody (Chemicon, Temecular, CA). The molecular size of ENT1 and β-actin are 40 and 43 kDa, respectively. Optical density values of the ENT1 bands were normalized to those of β-actin.

2.5. High affinity [^{3}H]NBMPR binding

Crude membranes were prepared as described for Western blotting. [^{3}H]NBMPR binding assays were performed at room temperature with 5 mM sodium phosphate (pH 8). Incubations were initiated by adding an aliquot of microsomal membranes of HASMCs (300 μg) to 0.5 nM [^{3}H]NBMPR and were terminated after 30 min by rapid filtration with Whatman GF/B filters, which were then washed twice with 5 ml ice-cold 5 mM sodium phosphate (pH 8). To determine the IC_{50} of thiazolidinediones on inhibition of [^{3}H]NBMPR binding, various concentrations of drugs (0–30 μM) were included with 0.5 nM [^{3}H]NBMPR during the 30 min incubation with microsomal membranes. The bound and the free [^{3}H]NBMPR were then separated similarly by rapid filtration. The radioactivity retained on the filters was counted by a β-scintillation counter after dissolving in Liquiscint (Amersham Biosciences, Piscataway, NJ). Nonspecific binding of [^{3}H]NBMPR was determined in the presence of 10 μM NBMPR.

2.6. Materials

All chemicals were purchased from Sigma–Aldrich (St. Louis, MO). [^{3}H]Adenosine and [^{3}H]uridine was from Amersham Biosciences (Piscataway, NJ). [^{3}H]NBMPR was from Moravek Biochemicals (Brea, CA). Cell culture media and supplements were from Invitrogen (Grand Island, NY).

2.7. Statistical analysis

Adenosine uptake data were expressed as means ± S.E.M. of three experiments performed in triplicate. Student's *t*-test and analysis of variance were used for paired and multiple variants, respectively. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of thiazolidinediones on ENT1 in HASMCs

It has been previously shown that [^{3}H]adenosine transport in HASMCs was mediated by the NBMPR-sensitive ENT1 and glucose treatment up-regulated ENT1 activity with a parallel increase in ENT1 protein and message. As thiazolidinediones bind to the nuclear receptor, PPARγ to regulate the transcription of a multitude of genes [20–23], we determined whether chronic exposure of HASMC to thiazolidinediones would affect ENT1 expression in HASMCs. As shown in Fig. 1A, all thiazolidinediones tested had no significant effect on NBMPR-sensitive [^{3}H]adenosine uptake (ENT1 activity) of HASMCs. Also, there was also no change in the mRNA and protein levels of ENT1 under these conditions (Fig. 1B and C). Glucose (25 mM) up-regulated ENT1 activity, mRNA and protein levels of HASMCs (Fig. 2A). However, thiazolidinediones had no effect on glucose stimulation of ENT1 activity, mRNA (Fig. 2B) and protein (Fig. 2C). Furthermore, the PPARγ antagonist, GW9662 (100 μM) also had no effect on glucose up-regulation of ENT1 (data not shown).

Adenosine is a vasodilator and thiazolidinediones have been shown to lower blood pressure in vivo [2,24,25]. Therefore, we tested whether the thiazolidinediones could directly affect ENT1 activity. It was found that troglitazone caused a concentration-dependent inhibition of [^{3}H]adenosine uptake of ENT1 in HASMCs with an IC_{50} of 2.35 ± 0.35 μM (Fig. 3). In contrast, pioglitazone and ciglitazone had minimal effects on [^{3}H]adenosine uptake in HASMCs (Fig. 3). Pioglitazone and ciglitazone at 30 μM inhibited [^{3}H]adenosine uptake of ENT1 by 13 and 8%, respectively. Similar results were obtained when the drugs were pre-incubated with cells for 30 min before the assay of [^{3}H]adenosine uptake. Equilibrative nucleoside transporters are broadly selective. As shown in Fig. 4, troglitazone, but not pioglitazone and ciglitazone also inhibited the [^{3}H]uridine uptake by HASMCs with a similar IC_{50} of 4.38 ± 0.34 μM. High affinity [^{3}H]NBMPR binding has been used for assessing the number of ENT1 molecules and the affinity of NBMPR to ENT1. It was found that troglitazone inhibited [^{3}H]NBMPR binding in a dose-dependent manner with an IC_{50} of 3.99 ± 0.57 μM (Fig. 5). The structural difference between troglitazone and other thiazolidinediones is that the former contains the Vitamin E moiety. However, Vitamin E did not affect the [^{3}H]adenosine uptake and [^{3}H]NBMPR binding of HASMCs (Fig. 6).

3.2. Kinetics of troglitazone inhibition of adenosine transport

The kinetic mechanism by which troglitazone inhibited adenosine transport was studied. As shown in Fig. 7A, troglitazone decreased the apparent K_m of [^{3}H]adenosine uptake without change on V_{max}. The apparent K_m values (μM) were 26.9 ± 0.89, 33.19 ± 4.11, 52.25 ± 11.14, and 364.99 ± 140.18, and the V_{max} values (pmol(mg protein · min)) were 30.27 ± 0.32, 29.19 ± 1.10, 30.31 ± 1.98, and 30.14 ± 4.13 in the presence of 0, 0.1, 1, 10 μM troglitazone, respectively. 1/[S] versus 1/*V* plots of each concentra-

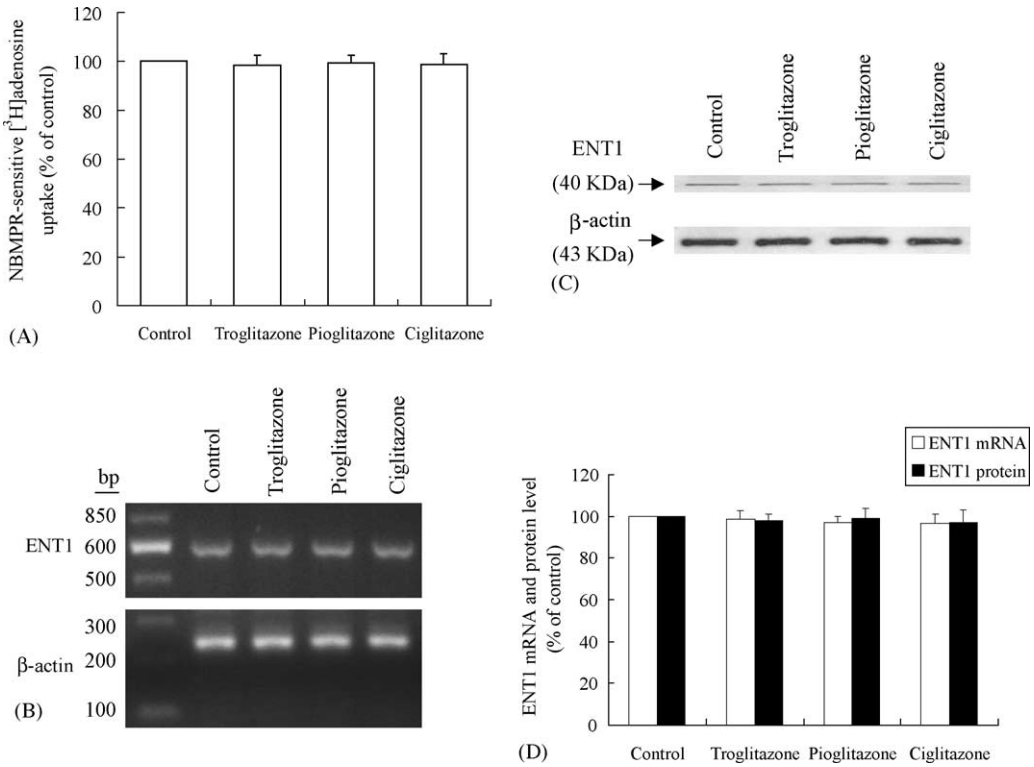

Fig. 1. Effects of chronic exposure of HASMCs to thiazolidinediones on ENT1 expression. Serum starved HASMCs were incubated without (control) and with 10 μM troglitazone, pioglitazone or ciglitazone for 48 h. Drugs were removed by washing the cells with Na^+-free buffer before the assay of [^{3}H]adenosine uptake activity and RNA isolation. (A) NBMPR-sensitive [^{3}H]adenosine uptake (10 μM, 2 μCi/ml, ± 10 μM NBMPR) in control and thiazolidinediones treated cells was measured at room temperature for 1 min. (B) Semi-quantitative RT-PCR analysis of ENT1 mRNA expression in HASMCs with reference to β-actin. (C) Western blot analysis of ENT1 protein expression in HASMCs with reference to β-actin. (D) Bar graph shows the amount of ENT1 mRNA (□) and protein (■) as normalized to β-actin. Values were means ± S.E.M. of three experiments.

tion-dependence curve showed that the slopes of the linear plots increased with increasing concentration of troglitazone (Fig. 7B). These slopes of the linear plots were then plotted against troglitazone concentrations (Fig. 7B, insert), revealing the K_i value of 2.23 ± 0.29 μM (n = 3).

3.3. Effects of troglitazone on recombinant ENT1 and ENT2

To test whether troglitazone is a general inhibitor of ENT isoforms, we studied the effects of troglitazone on recombinant hENT1 and hENT2, which have been transfected into the nucleoside transporter-deficient cell line, PK15NTD [26]. Consistent with the results from HASMCs, troglitazone inhibited recombinant ENT1 in a dose-dependent manner with an IC_{50} of 5.17 ± 0.92 μM (Fig. 8). Recombinant ENT2, however, was relatively resistant to troglitazone. 30 μM of troglitazone, which completely inhibited recombinant ENT1, only inhibited recombinant ENT2 by 12%.

4. Discussion

Nucleoside transporters play crucial roles in adenosine homeostasis and are important in modulating the effects of adenosine on vascular SMCs. Recent studies have shown that inhibition of ENT1 increases extracellular adenosine concentrations and this causes vasodilation and inhibits vascular SMC proliferation [27–29]. In diabetics, changes in expression of ENT1 are tissue specific. ENT1 expression in heart, liver and kidney of streptozotocin-induced diabetic rats are decreased [15]. However, ENT1 message is increased in freshly isolated human umbilical artery SMCs [16] but is decreased in the umbilical vein endothelial cells from gestational diabetic pregnancies [30], when the effects are compared to the corresponding cells isolated from normal pregnancies. High glucose at 25 mM which mimics glucose levels in diabetics also decreases adenosine transport of ENT1 in human umbilical vein endothelial cells [17] but increases adenosine transport of ENT1 in HASMCs [18]. However, whether insulin could reverse the effect of diabetics on ENT1 expression in tissues and/or cells is controversial. Administration of insulin to streptozotocin-induced diabetic rats was unable to restore the decreased ENT1 expression in heart, liver and kidney [15]. In contrast, Aguayo and co-workers demonstrated that insulin inhibited the elevated ENT1 expression in human umbilical artery SMCs from diabetic pregnancy via activation of adenylyl cyclase [16]. This discrepancy may be due to differences in species (rat versus human) and/or models (animal versus primary cultured cells). To our knowledge,

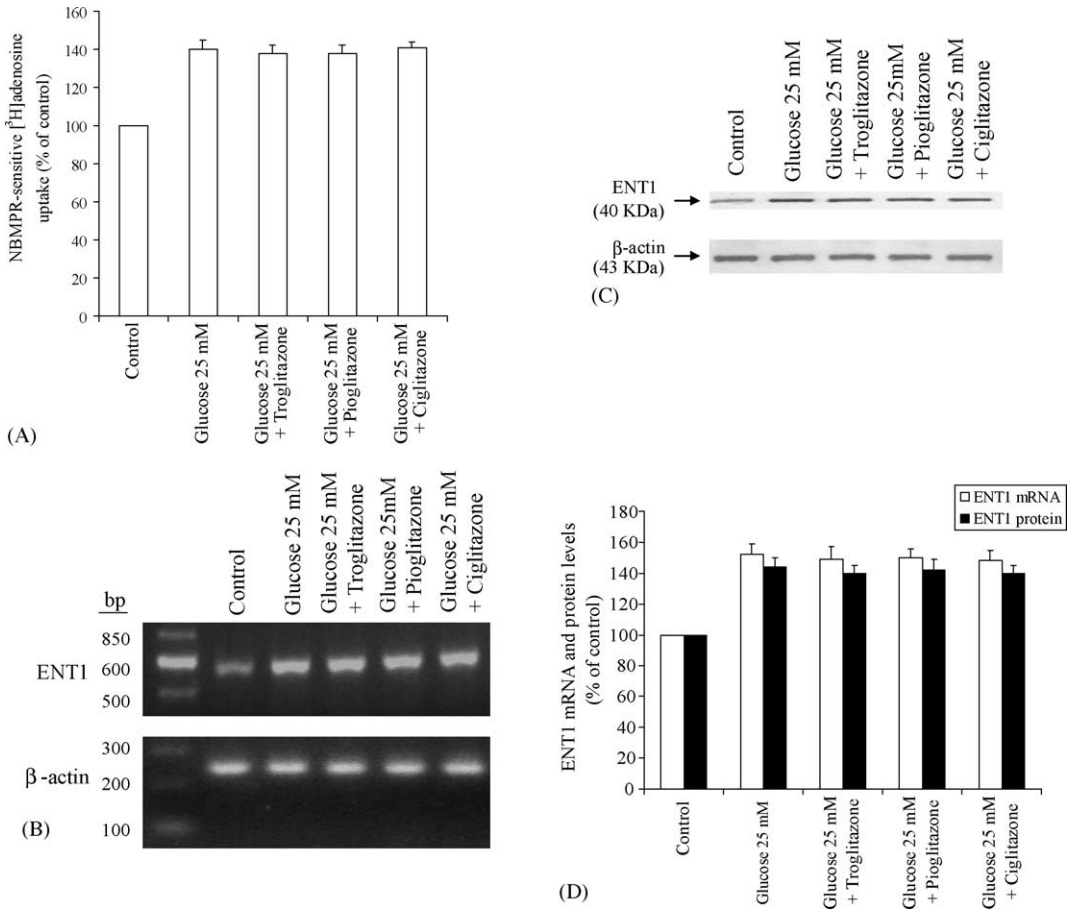

Fig. 2. Effects of chronic exposure to thiazolidinediones on ENT1 expression of HASMCs grown in the presence of 25 mM glucose. Serum starved HASMCs were incubated with 25 mM glucose in the absence (control) and in the presence of 10 μM troglitazone, pioglitazone or ciglitazone as indicated for 48 h. Drugs were removed by washing the cells with Na^+-free buffer before the assay of [^{3}H]adenosine uptake activity and RNA isolation. (A) NBMPR-sensitive [^{3}H]adenosine uptake (10 μM, 2 μCi/ml, ±10 μM NBMPR) in control and thiazolidinediones treated cells was measured at room temperature for 1 min. (B) Semi-quantitative RT-PCR analysis of ENT1 mRNA expression in HASMCs grown in the presence of 25 mM glucose and 10 μM thiazolidinediones with reference to β-actin mRNA expression. (C) Western blot analysis of ENT1 protein expression in HASMCs grown in the presence of 25 mM glucose and 10 μM thiazolidinediones with reference to the amount of β-actin. (D) Bar graph shows the amount of ENT1 mRNA (□) and protein (■) as normalized to β-actin. Values were means ± S.E.M. of three experiments.

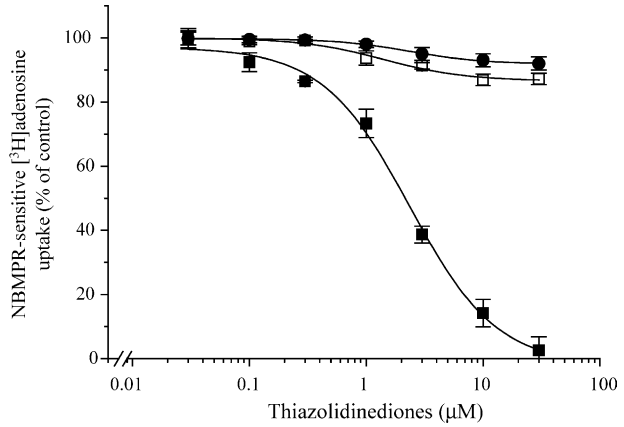

Fig. 3. Effects of thiazolidinediones on [^{3}H]adenosine uptake by HASMCs. NBMPR-sensitive [^{3}H]adenosine uptake (10 μM, 2 μCi/ml, ±10 μM NBMPR) was measured at room temperature for 1 min in the presence of various concentrations of troglitazone (■), pioglitazone (□) or ciglitazone (●). Troglitazone was added simultaneously with [^{3}H]adenosine. Values were means ± S.E.M. of three experiments performed in triplicate.

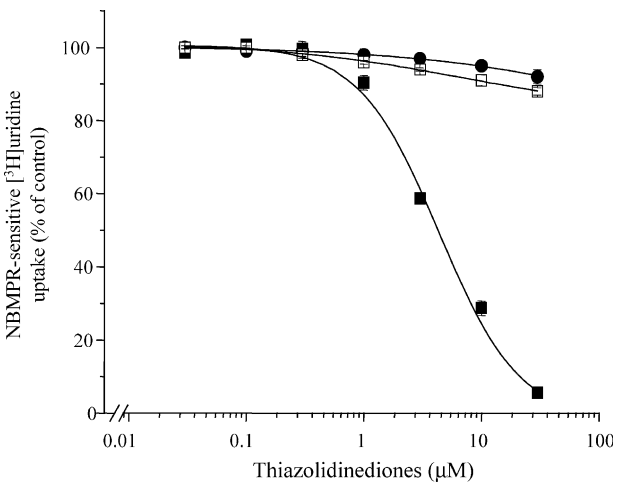

Fig. 4. Effects of thiazolidinediones on [^{3}H]uridine uptake by HASMCs. NBMPR-sensitive [^{3}H]uridine uptake (10 μM, 2 μCi/ml, ±10 μM NBMPR) was measured at room temperature for 1 min in the presence of various concentrations of troglitazone (■), pioglitazone (□) or ciglitazone (●). Values were means ± S.E.M. of three experiments performed in triplicate.

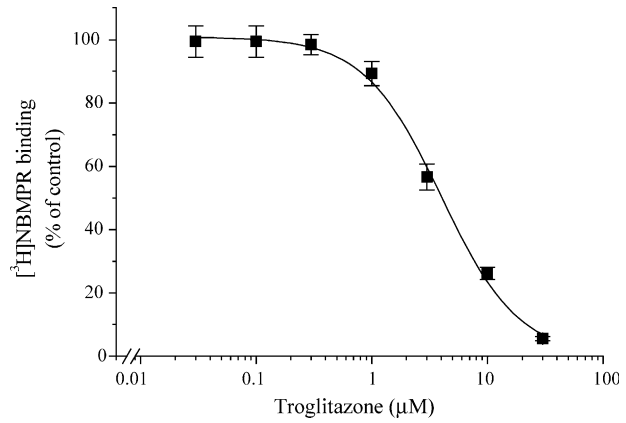

Fig. 5. Effect of troglitazone on [^{3}H]NBMPR binding to HASMCs. Microsomal membranes of HASMCs were incubated with various concentrations of troglitazone and 0.5 nM [^{3}H]NBMPR for 30 min. High affinity [^{3}H]NBMPR binding was determined as described in Materials and Methods. Values were means ± S.E.M. of three experiments performed in triplicate.

the effects of antidiabetic agents other than insulin, on ENT1 have not been reported. Although chronic incubation of thiazolidinediones did not change ENT1 expression in HASMCs, the present study showed that a thiazolidinedinone, troglitazone is a novel competitive inhibitor of ENT1.

Thiazolidinediones are agonists for PPARγ, which is a nuclear receptor predominantly expressed in adipose tissue, where it promotes adipocyte differentiation and regulates expression of genes involved in glucose and fatty acid metabolism [31]. Recently, the existence of PPARγ has been reported in vascular SMCs [32,33]. PPARγ activation has been shown to inhibit vascular SMC proliferation and migration [5,8,20], suggesting that PPARγ may play a role in limiting the arterial remodeling that occurs in response to hypertension, atherosclerosis or restenosis. It has been shown that PPARγ activation in vascular SMCs inhibits the expression of matrix metalloproteinases [20], thromboxane receptor [21], platelet-derived growth factors-α receptor [22] and angiotensin II type I receptor [23]. We have previously shown that chronic incubation of HASMCs with high glucose increases the amount of ENT1 message and thus ENT1 is regulated at the transcriptional level [18]. Given that PPARγ regulates various genes at the transcription level, we examined whether thiazolidinediones affected ENT1 expression in HASMCs. Exposure of thiazolidinediones to HASMCs, either in the presence or the absence of 25 mM glucose, for 48 h did not affect the ENT1 abundance in HASMCs (Figs. 1 and 2). Therefore, our data suggested that PPARγ was involved neither in the regulation of the basal expression of the ENT1 nor the glucose up-regulated ENT1 expression.

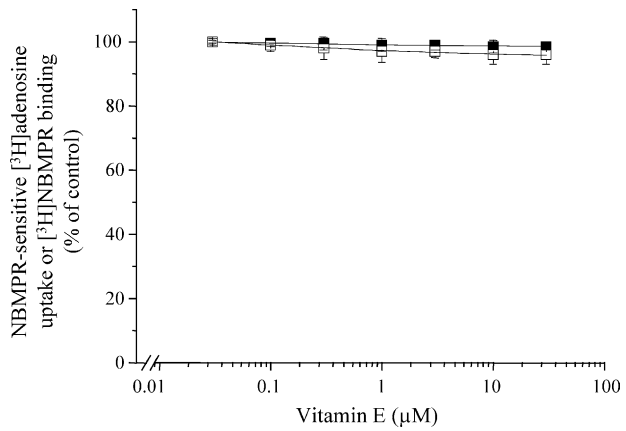

Fig. 6. Effects of Vitamin E on [^{3}H]adenosine uptake and [^{3}H]NBMPR binding of HASMCs. [^{3}H]Adenosine uptake (10 μM, 1 min, 2 μCi/ml, ±10 μM NBMPR) (■) and [^{3}H]NBMPR (0.5 nM) binding (□) were measured at room temperature in the presence of different concentrations of Vitamin E. Values are means ± S.E.M. of three experiments performed in triplicate.

Although all thiazolidinedione analogs have similar functions as insulin sensitizers whose effects are PPARγ-dependent, each analog might have unique PPARγ-independent effects on the vasculature and such effects do not modify gene transcription. For example, troglitazone and rosiglitazone inhibit voltage-dependent K^+ channels and nonselective cation channels in vascular SMCs, but they have opposite effects on Ca^{2+}-activated K^+ channels [6,7,34]. Troglitazone differs from other thiazolidinediones in that it has a Vitamin E moiety as its side-chain and thus possesses radical scavenging ability similar to Vitamin E [35,36]. This property of troglitazone is accounted for the improvement of the reduced skin blood flow in diabetic rats [37]. However, not all effects of troglitazone are attributed to the radical scavenger actions of Vitamin E. Troglitazone, but not Vitamin E, inhibits the DNA synthesis in vascular SMCs [38]. Similarly, our results showed that Vitamin E did not affect ENT1 activity (Fig. 6) and thus, the competitive inhibition of troglitazone on ENT1 was Vitamin E-independent. The K_i of inhibition of troglitazone on ENT1 was 2.23 ± 0.29 μM (Fig. 7). This K_i value correlates well with potential pharmacological concentrations of troglitazone in vivo. It has been reported that 2.5–3.5 h after 200 mg troglitazone, a dose that improves glycaemic control and insulin sensitivity, the plasma concentration of troglitazone in human is 11.3–15.9 μM [39]. Therefore, troglitazone is a potential in vivo inhibitor of ENT1 at pharmacological concentrations.

Recently, it was shown that a wide variety of protein kinase inhibitors are also inhibitors of ENT1 and their actions on ENT1 are kinase-independent [40,41]. However, whether these protein kinase inhibitors affect ENT2 has not yet been clarified. We have generated a nucleoside transporter-deficient cell line, PK15NTD, and have stably expressed ENT1 and ENT2 in this cell model [26]. Therefore, we examined the sensitivities of ENT1 and ENT2 to troglitazone in PK15NTD/ENT1 and PK15NTD/ENT2 cells. Our results showed that troglitazone is a competitive

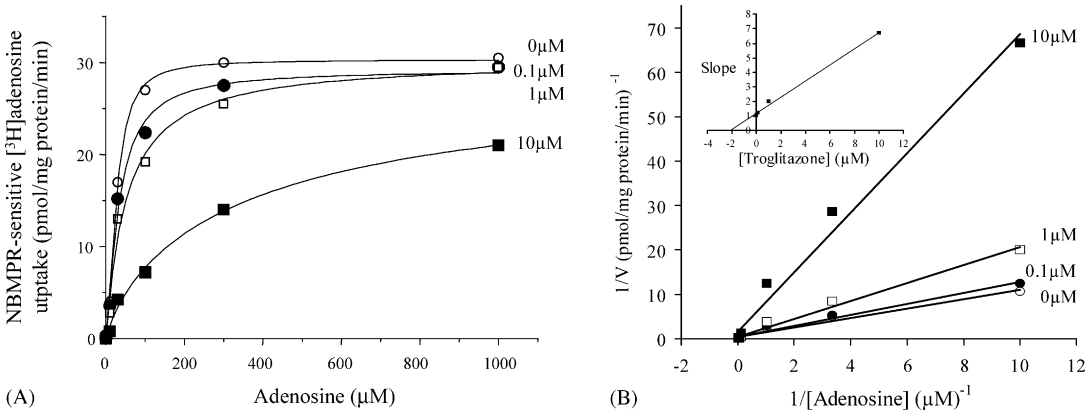

Fig. 7. Competitive inhibition of [^{3}H]adenosine uptake by HASMCs by troglitazone. (A) Concentration dependence of [^{3}H]adenosine uptake (0–1000 μM) was measured at room temperature for 1 min in the presence of various troglitazone concentration (0, 0.1, 1, 10 μM). (B) 1/[S] vs. 1/*V* plots of each concentration-dependence curve in A. The lines are best-fitted lines drawn with Origin software. The inset shows the plot with slopes (obtained from each linear plot) vs. [Troglitazone]. $K_i = 2.23 \pm 0.29$ μM ($n = 3$, x-intercept = $-K_i$).

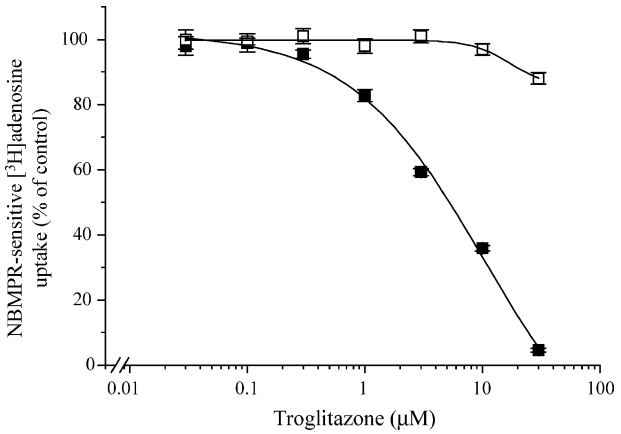

Fig. 8. Effects of troglitazone on PK15NTD/ENT1 and PK15NTD/ENT2. NBMPR-sensitive [^{3}H]adenosine uptake (10 μM, 2 μCi/ml, ±10 μM NBMPR) by PK15NTD cells stably transfected with either ENT1 (■) or ENT2 (□) was measured at room temperature for 2 min in the presence of various concentrations of troglitazone. Values were means ± S.E.M. of three experiments performed in triplicate.

inhibitor of ENT1 (Fig. 7). In contrast, ENT2 is resistant to troglitazone up to 10 μM (Fig. 8).

In conclusion, thiazolidinediones have no effect on the basal expression of ENT1 and on the glucose up-regulated ENT1 expression in HASMCs. However, a thiazolidinediones, troglitazone, is a competitive inhibitor of ENT1. The inhibition of ENT1 activity by troglitazone may influence the availability of adenosine in the vicinity of adenosine receptors and subsequently affect vascular function.

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