Resveratrol inhibits 11β-hydroxysteroid dehydrogenase type 1 activity in rat adipose microsomes

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Abstract

It has been suggested that resveratrol, a polyphenol in wine, can regulate adiposity because it decreases adipose deposition in mice and rats; however, the mechanism underlying this effect has not been fully clarified. In humans and rodents, 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is expressed in liver and adipose tissue. 11β-HSD1 converts inactive glucocorticoid into active glucocorticoid in adipocytes. Activated glucocorticoid plays an important role in the pathogenesis of central obesity. The objective of this study was to investigate the effects of resveratrol on 11β-HSD1 activity in rodent adipose tissue. 11β-HSD1 activity in microsomes from rat mesenteric adipose depots and 3T3-L1 adipocytes was determined in the presence of 11-dehydrocorticosterone with or without varying concentrations of resveratrol. Significant inhibition of 11β-HSD1 by resveratrol was observed in rat adipose microsomes and 3T3-L1 adipocytes within 10 min. Time- and dose-dependent effects were also observed. The 11β-HSD1 activity by resveratrol was also inhibited in rat epididymal adipose tissue, and this inhibition was not recovered by estrogen receptor blockers. The kinetic study revealed that resveratrol acted as a non-competitive inhibitor of 11β-HSD1. $K_i$ and $IC_{50}$ values of resveratrol were 39.6 and 35.2 μM respectively. Further, resveratrol did not affect the activities of 11β-HSD2 and hexose-6-phosphate dehydrogenase. These results suggest that the most likely mechanism of 11β-HSD1 inhibition by resveratrol is via interaction between resveratrol and 11β-HSD1 enzyme, rather than via a transcriptional pathway. We demonstrated that the antiobesity effects of resveratrol may partially be attributed to the inhibition of 11β-HSD1 activity in adipocytes.

Keywords
- 11β-HSD1
- resveratrol
- antiobesity effect
- adipocyte

Introduction

Glucocorticoids affect a wide variety of physiological functions, including glucose and lipid metabolism, cell growth, immune modulation, and anti-inflammatory responses. They also play a pivotal role in the local regulation of adipose tissue function, development, and distribution. Intracellular glucocorticoid levels are regulated by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) and 11β-HSD2. 11β-HSD1, which is abundantly expressed in the adipose tissue and in the liver, acts as an oxoreductase converting inactive cortisone into bioactive cortisol in humans (11-dehydrocorticosterone into corticosterone in rodents) (Pereira et al. 2012),...
whereas 11β-HSD2 catalyzes the reverse reaction to 11β-HSD1, mainly in the kidney. Recently, evidence has been accumulated that intracellular glucocorticoid amplification in adipocytes by 11β-HSD1 contributes to central obesity and, consequently, promotes metabolic diseases in experimental animals (Masuzaki et al. 2001) and humans (Buijalska et al. 1997, Wake et al. 2003). 11β-HSD1 knockout mice have demonstrated reduced visceral fat depot and improved insulin sensitivity (Kotelevtsev et al. 1997). Pharmacological inhibition of 11β-HSD1 by an 11β-HSD1 inhibitor such as carbenoxolone (CBX), in high-fat diet-induced obese mice and type 2 diabetes animal models, improved obesity and glucose tolerance (Livingstone & Walker 2003, Taylor et al. 2008). Therefore, suppression of local glucocorticoid accumulation in tissues through inhibition of 11β-HSD1 may be an effective treatment for central obesity and metabolic syndrome. A number of chemical compounds targeting 11β-HSD1 inhibition have been explored, and 11β-HSD1 inhibitor has been shown to be effective in improving metabolic syndrome in humans (Boyle 2008, Anagnostis et al. 2013).

11β-HSD1 requires NADPH as a co-enzyme for full activation of the 11-oxoreductive reaction of 11-dehydrocorticosterone and cortisone. NADPH is supplied to 11β-HSD1 by hexose-6-phosphate dehydrogenase (H6PD), which is colocalized with 11β-HSD1 in the intralumen of the endoplasmic reticulum (Hewitt et al. 2005). H6PD generates NADPH and 6-phosphogluconate from NADP and glucose-6-phosphate (G6P). The transport of G6P from the cytosol into the endoplasmic reticulum is facilitated by G6P transporter (G6PT), which is found on the surface of the endoplasmic reticulum. These enzymes and carrier proteins are compartmentalized in the endoplasmic reticulum; thus, 11β-HSD1 activity substantially depends on the activities and functions of H6PD and G6PT.

Recently, we found that an endogenous steroid hormone, 17β-estradiol (E2), inhibited 11β-HSD1 activity in rodent adipocytes in a non-competitive manner (Tagawa et al. 2009). E2 has phenolic and alcoholic hydroxyl groups at the C-3 and C-17β positions respectively. These hydroxyl groups at the C-3 and C-17 positions of E2 were indispensable for the inhibitory effect of 11β-HSD1 activity, which was revealed by the structure–activity relationship of those groups. Further, the distance between these two hydroxyl groups of E2 was 12.12 Å (Kiyonaga et al. 2012; Fig. 1B), whereas resveratrol (3, 4′, 5-trihydroxystilbene) bears three hydroxyl groups. The distances between the two hydroxyl groups at C-4′ and C-3 and C-4′ and C-5 positions, which were calculated using MOPAC PM5/Cache Worksystem version 6.1, were 12.57 and 11.78 Å respectively (Fig. 1A). Both these distances between the two hydroxyl groups in resveratrol approximated those in the two hydroxyl groups of E2.

More recently, we have also demonstrated that 2-arylbenzofuran derivatives bearing two hydroxyl groups in the molecule (Fig. 1C and D), the distances of which were ~12 Å (similar to E2), inhibited 11β-HSD1 activity in a non-competitive manner in rodent adipocytes (Kiyonaga et al. 2012). These findings prompted us to examine whether the compound bearing a structural resemblance to the spatial configuration of the two hydroxyl groups in E2 inhibits 11β-HSD1 activity. Therefore, we evaluated resveratrol as an inhibitor of 11β-HSD1.

Some natural compounds and their derivatives, such as glycyrrhetinic acid, emodine, and CBX, have potential 11β-HSD1 inhibition (Boyle 2008, Feng et al. 2010). Resveratrol is a natural polyphenol and a stilbene-type phytoalexin produced by plants in response to environmental stress. Similar to most polyphenols, resveratrol has antioxidant activity. It is commonly found in fresh grape skins, red wine (pinot noir), boiled peanuts, and blueberries (dry sample) at 5–10 mg/100 g, 10.5 mg/l, 0.52 mg/100 g, and 0.4 mg/100 g respectively (Chachay et al. 2011). As an ingredient of red wine, resveratrol explains the epidemiological phenomenon called the ‘French paradox’, in which red wine consumption is associated with a low incidence of cardiovascular disease (Petrovski et al. 2011, Smoliga et al. 2011, Turan et al. 2012). In recent years, resveratrol has been shown to
activate AMPK and Sirt1 and to interact with a number of receptors, kinases, and other enzymes that contribute to its biological effects (Turan et al. 2012). Most recently, resveratrol has been of interest because of its various favorable pharmacological effects of improving longevity (Howitz et al. 2003, Picard et al. 2004, Milne et al. 2007). Accumulating evidence suggests that resveratrol has the potential to prevent obesity, diabetes, cardiovascular diseases, and a variety of human diseases (Szkudelska & Szkudelski 2010, Petrovski et al. 2011). Several mechanisms, such as activation of Sirt1 or AMPK, have also been proposed to explain these effects of resveratrol (Turan et al. 2012); however, the impact of resveratrol on 11β-HSD1 in adipose tissue has not been demonstrated. Therefore, in this study, we investigated the effect of a phytoestrogen, resveratrol, on the activities of 11β-HSD1, 11β-HSD2, and H6PD using rat adipose and liver tissues and 3T3-L1 adipocytes.

Materials and methods

Materials

Steroid standards were obtained from Steraloids, Inc. (Newport, RI, USA); 3-(N-morpholino)propanesulfonic (MOPS) acid from Sigma; NADP, NADPH, G6P, and tamoxifen (TAM) from Wako Pure Chemical Industries (Osaka, Japan); and ICI 182 780 from Tocris Bioscience (St Louis, MO, USA). Other reagents were of analytical grade and used without further purification.

Animals

Male Wistar rats (10 weeks old) were obtained from SLC Co. Ltd. (Shizuoka, Japan). They were housed under a controlled 12 h light:12 h darkness cycle (light from 0700 to 1900 h) with a room temperature of 23±1 °C and humidity at 55±5%. The rats had access to food ad libitum. After a resting period of 1 week, the animals were killed by bleeding from the abdominal aorta under light ether anesthesia. The experimental procedures were approved by Kobe Pharmaceutical University Animal Care and Use Committee.

Culture and differentiation of 3T3-L1 cells and microsome preparation

Differentiated 3T3-L1 adipocytes and intact microsomes from mesenteric fat pads and the liver and kidney of rats were prepared according to the method described elsewhere (Tagawa et al. 2009). Because it has been reported that there is no isozyme for 11β-HSD1 in liver or adipose tissue from humans and rodents, we confirmed this in this study. H6PD also has no isozyme in humans or rodents. Likewise, we probed for H6PD activity in liver and adipose tissue microsomes. Mesenteric fat pads, liver, or kidney from ten Wistar rats were obtained. These tissues were combined to prepare microsomes of adipose tissue, liver, or kidney microsomes respectively. The integrity of the microsomal membrane was inferred from the latency of glucose dehydrogenase activity, which was found to be >95% in all the preparations assayed.

Measurement of 11β-HSD1 activity

11β-HSD1 activity (11-oxoreductase) was measured using i) rat microsomes from adipose tissue (mesenteric fat depots) or liver, ii) 3T3-L1 adipocytes, and iii) rat epididymal adipose tissue according to the method previously described with minor modification (Tagawa et al. 2009). Each assay was performed in duplicate or multiplicate (number of data (n): 3–5) using 10 μl microsomes, 3T3-L1 adipocytes in six-well plates, or a slice of rat epididymal adipose tissue.

i) An aliquot (10 μl) of microsomes (adipose tissue 4.0 mg protein/ml; liver 0.16 mg protein/ml) was added to MOPS buffer (100 mM KCl, 20 mM NaCl, and 20 mM MOPS, pH 7.4) containing NADPH (1 mM) and 11-dehydrocorticosterone (1 μM) with or without various concentrations of resveratrol or the test compound and was incubated at 37 °C for 40 min (adipose tissue) or 20 min (liver). The final concentration of the DMSO solution, which dissolved the substrate and the reactants, was below 0.05-0.1% in the incubation medium. After extraction of the reaction mixture with dichloromethane and evaporation in vacuo, corticosterone concentrations were measured by reverse-phase semi-micro HPLC in an isocratic mode (water:methanol=45:55, v/v%) with u.v. detection.

ii) 3T3-L1 adipocytes in six-well plates were incubated in medium containing 1 μM 11-dehydrocorticosterone with or without the test compound at 37 °C for 10 min in a humidified atmosphere in the presence of 10% CO₂. After extraction of the medium, corticosterone concentrations were measured by the above-mentioned HPLC method.

iii) A slice of rat epididymal adipose tissue (0.054–0.091 g) was incubated in DMEM containing 25 μM resveratrol and 1 μM 11-dehydrocorticosterone with or without
the test compound at 37 °C for 40 min. After extraction of the reaction mixture with dichloromethane and evaporation in vacuo, triglycerides in the residues were removed by washing with mixed solution of 1 ml heptane and 1 ml methanol–water (7:2) three times. The upper (heptane) layer was discarded. The lower (methanol) layer was collected and combined, and the extract was washed with 2 ml water. The solvent was evaporated to dryness. The corticosterone concentrations in the extract were measured by the above-mentioned HPLC method.

Under these conditions, the appearance of corticosterone was linear for 1 h using microsomes from adipose tissue and the liver, 3T3-L1 adipocytes, and adipose tissue (data not shown). The intra-assay coefficient of variation (CV) for the measurement of 11β-HSD1 activity in controls (without resveratrol) using microsomes, 3T3-L1 adipocytes, and adipose tissues were 1.9% (n = 5), 2.8% (n = 5), and 6.2% (n = 5) respectively.

**Measurement of 11β-HSD2 activity**

11β-HSD2 activity (dehydrogenase) in microsomes from rat kidney was assayed in MOPS buffer containing 2 mM NAD and corticosterone (1 mM) with or without various concentrations of the test compound. The reaction was started by the addition of 10 μl microsomes (2.3 mg protein/ml), and the reactants were incubated at 37 °C for 20 min. The reaction mixture was extracted with dichloromethane and evaporated in vacuo. Subsequently, 11-dehydrocorticosterone concentrations were measured by the above-mentioned HPLC method. The appearance of 11-dehydrocorticosterone was linear for 1 h (data not shown). The intra-assay CV for the measurement of 11β-HSD2 activity in controls (without resveratrol) using microsomes was 4.8% (n = 5). Each assay was performed in quadruplicate using 10 μl microsomes.

**Measurement of H6PD activity**

H6PD activity was measured by fluorometric detection of NADPH produced in the MOPS buffer containing 6 mM G6P and 1 mM NADP at room temperature for 60 min. The reaction was started by the addition of intact microsomes (10 μl, adipose tissue: 1.5 mg protein/ml; liver: 2.4 mg protein/ml) to the cuvette in triplicate. The compounds evaluated were dissolved in DMSO and added to the MOPS buffer at a final concentration of DMSO below 0.05%. Changes in fluorescent intensity (340 nm excitation and 460 nm emission wavelengths) were measured using a Shimadzu RF 510 fluorescence spectrophotometer (Shimadzu, Kyoto, Japan). NADPH concentration was measured by a standard curve obtained from pure NADPH. The intra-assay CV for measurement of H6PD activity in the controls (without resveratrol) was 2.0% (n = 5). Each assay was performed in duplicate or multiplicate (n = 3 or 4) using 10 μl microsomes.

**Kinetic assay**

Enzyme reactions were performed in 1 ml MOPS buffer containing 0.25–1 μM 11-dehydrocorticosterone, 6 mM G6P, 1 mM NADP, and 0.35 units/ml G6PD at 37 °C for 60 min. If necessary, 1–25 μM test compound was added to the buffer. The reaction was initiated by the addition of microsomes (10 μl, adipose tissue: 4.0 mg protein/ml; liver: 0.2 mg protein/ml). Steroid concentrations were measured by the above-mentioned HPLC method. The linearity of enzyme activity vs time and protein concentration was confirmed. Michaelis constant (K_m) and inhibition constant (K_i) value estimations were averaged from Lineweaver–Burk plots and Dixon plots using triplicate samples respectively. The 50% inhibitory concentration (IC_50) values were obtained from activity (%) vs compound concentration plots.

**Statistical analysis**

Data are expressed as the mean ± S.E.M. Using the computer software Statflex ver. 6 (Artech Co., Ltd., Osaka, Japan), statistical analyses were performed by one-way ANOVA followed by Dunnett’s test for multiple comparisons if the overall P values were significant. P<0.05 was considered significant.

**Results**

**Resveratrol inhibited 11β-HSD1 in rat mesenteric fat pads**

To explore whether resveratrol inhibits 11β-HSD1 activity in a cell-free system, microsomes from rat mesenteric fat pads were incubated with and without resveratrol (50 μM; Fig. 2A). E_2 (50 μM) and CBX (50 μM) were used as positive controls. Resveratrol inhibited 11β-HSD1 reductase activity (conversion of 11-dehydrocorticosterone into corticosterone) significantly by 47.7% (0.85 ± 0.04 nmol/h per mg protein) compared with the control (1.77 ± 0.03 nmol/h per mg protein, P<0.001, one-way ANOVA; P<0.01, Dunnett’s test). Similarly, E_2 and
Resveratrol inhibited 11β-HSD1 in rat adipose tissue and liver in a time- and dose-dependent manner

We next examined the time- and dose-dependent effects of 11β-HSD1 inhibition by resveratrol using rat adipose tissue and liver microsomes. Resveratrol inhibited 11β-HSD1 activity significantly in a time-dependent (Fig. 2B; 25 μM resveratrol, P<0.05–0.001) and dose-dependent (Fig. 2C; 1–10 μM resveratrol) manner (adipose tissue microsomes: P<0.001, one-way ANOVA; P<0.01, Dunnett’s test; liver microsomes: P<0.01, one-way ANOVA; P<0.01, Dunnett’s test).

ER blockers do not influence the inhibitory effect of resveratrol on 11β-HSD1 activity

Resveratrol has been reported to act as an agonist at the estrogen receptor (Bowers et al. 2000). Recently, it has been accepted that ERβ exists and functions as a non-traditional G-protein-coupled receptor at the plasma membrane. It has been suggested that such membrane receptors mediate rapid non-genomic steroid-signaling events (Pappas et al. 1995, Revankar et al. 2005). Therefore, we evaluated the involvement of ERs in the 11β-HSD1 inhibitory effect of resveratrol on rat adipose tissue using the ER antagonists ICI 182 780 and TAM. A slice of rat epididymal adipose tissue was incubated in DMEM containing 25 μM resveratrol and 1 μM 11-dehydrocorticosterone with or without 1 μM ICI at 37 °C for 40 min. Epididymal adipose tissues were also incubated in DMEM containing 10 μM TAM prior to the addition of resveratrol for 10 min. Subsequently, resveratrol and 11-dehydrocorticosterone were added to the tubes and incubated at 37 °C for 40 min. A significant inhibition of 51.6% (P<0.01, from 0.707±0.085 to 0.364±0.028 nmol/h per g tissue) compared with the control (vehicle without ER antagonists) was observed by resveratrol in the epididymal adipose tissue and in the microsomes of mesenteric adipose tissue, and this inhibition was not recovered in the presence of TAM (P<0.001, 48.7% inhibition from 0.639±0.045 to 0.311±0.007 nmol/h per g tissue) or ICI (P<0.05, 51.1% inhibition from 0.714±0.048 to 0.365±0.090 nmol/h per g tissue; Fig. 3).

Resveratrol did not affect 11β-HSD2 activity in rat kidney

The effect of resveratrol on 11β-HSD2 activity (conversion of corticosterone into 11-dehydrocorticosterone) was evaluated using rat kidney microsomes. Resveratrol (1–10 μM) did not affect 11β-HSD2 activity (data not

CBX inhibited 11β-HSD1 by 22.0% (0.39±0.05 nmol/h per mg protein) and 11.3% (0.20±0.01 nmol/h per mg protein) respectively (E2 and CBX; P<0.01, Dunnett’s test). Puerarin (a daidzein-8-C glucoside) is an isoflavonetype phytoestrogen that contains two hydroxyl groups whose distance is ~12 Å. However, because it also contains a bulky and hydrophilic β-D-glucopyranosyl group at C-8, the structure is different from resveratrol. Puerarin (50 μM) was incubated to verify the specificity of the effect of resveratrol on 11β-HSD1 as a negative control. Puerarin did not inhibit 11β-HSD1 activity.
Resveratrol inhibited 11β-HSD1 activity in a non-competitive manner

To substantiate the above-mentioned inhibitory effect of resveratrol on 11β-HSD1, we next conducted kinetic studies. Lineweaver–Burk plots, which distinguish the type of inhibition (Voet & Voet 2004), indicated that resveratrol acted as a non-competitive inhibitor of 11β-HSD1 because the slopes of the five lines (without compound and with different levels of resveratrol) were different, but all converged to the same point of one per substrate (x-) axis (Fig. 6A). The apparent $K_m$ and $V_{max}$ values for 11-dehydrocorticosterone with adipose tissue microsomes were $\approx 0.66 \mu M$ and $5.72 \text{ nmol/h per mg protein}$ respectively. The $K_m$ and $V_{max}$ values with liver microsomes were $\approx 0.66 \mu M$ and $42.5 \text{ nmol/h per mg protein}$ respectively. Dixon plots (Dixon 1953), which were obtained by plotting the values of reciprocal $V_{max}$ as a function of resveratrol concentrations, also showed that the inhibition type was non-competitive for resveratrol and yielded $K_i$ (Fig. 6B). $K_i$ and $IC_{50}$ of resveratrol in adipose tissue from rats were 39.6 and 35.2 $\mu M$ shown). CBX (10 $\mu M$), a positive control, inhibited 11β-HSD2 activity by 0.96% (from mean $\pm$ S.E.M. 11.8 $\pm$ 0.4 to 0.08 $\pm$ 0.01 nmol 11-dehydrocorticosterone/h per mg protein; $P<0.001$, one-way ANOVA; $P<0.01$, Dunnett’s test, data not shown).

Resveratrol did not affect H6PD activity in rat adipose tissue and liver

The effect of resveratrol on H6PD activity (conversion of NAD into NADPH) was evaluated using intact microsomes prepared from rat adipose or liver tissues. Resveratrol (1–50 $\mu M$) did not alter H6PD activity (Fig. 4).

Resveratrol inhibited 11β-HSD1 activity in 3T3-L1 adipocytes

To investigate the effect of resveratrol on 11β-HSD1 activity in adipocytes, differentiated 3T3-L1 adipocytes were incubated in DMEM containing 11-dehydrocorticosterone with resveratrol, CBX, and spironolactone (each concentration 50 $\mu M$; Fig. 5). Resveratrol significantly inhibited 11β-HSD1 activity by 68.7% (from mean $\pm$ S.E.M. 0.87 $\pm$ 0.05 to 0.66 $\pm$ 0.04 nmol corticosterone/h per mg protein) in 3T3-L1 adipocytes ($P<0.001$, one-way ANOVA; $P<0.01$, Dunnett’s test).
resveratrol increases apoptosis of adipocytes, decreases proliferation and differentiation of pre-adipocytes, reduces adipogenesis, and enhances lipolysis (Baile et al. 2011). However, the underlying mechanisms of those effects have not been fully elucidated. Some of the antiobesity effects of resveratrol have been partially explained due to its ability to increase the activation of Sirt1 and AMPK. These mechanisms of the antiobesity effects of resveratrol were mediated by the transcriptional pathway; however, here, the inhibition of 11β-HSD1 activity by resveratrol was observed within 10 min in a cell-free system using microsomes (Fig. 2). Occurrence of 11β-HSD1 inhibition within 10 min was also observed in living cells, 3T3-L1 adipocytes (Fig. 5). Further, kinetic study revealed that resveratrol inhibited 11β-HSD1 in a non-competitive manner (Fig. 6). These results suggest that 11β-HSD1 inhibition by resveratrol is via interaction between resveratrol and 11β-HSD1, rather than via the transcriptional pathway. Further study will be

respectively and were similar to those in liver (K\textsubscript{i}, 39.6 µM and IC\textsubscript{50}, 36.6 µM). Therefore, resveratrol showed a potent inhibitory effect on 11β-HSD1 activity.

Discussion

11β-HSD1 activity in adipocytes significantly contributes to the development of obesity; therefore, inhibition of 11β-HSD1 in adipose tissue is one of the keys to the prevention of and therapy for obesity. In this study, we demonstrated that resveratrol selectively inhibited 11β-HSD1 activity in rat adipose tissues, liver, and 3T3-L1 adipocytes. This study demonstrated an alternative antiobesity effect of resveratrol, preventing intracellular glucocorticoid reamplification by 11β-HSD1 in a non-transcriptional pathway.

Resveratrol has been suggested to be effective in preventing the development of obesity, which is a pathophysiological basis for various metabolic diseases including diabetes, cardiovascular disease, and dyslipidemia. Resveratrol has also been shown to have multiple actions on antiobesity. For instance, in adipocytes,
needed to establish the interaction between resveratrol and 11β-HSD1.

It has been reported that non-genomic biological effects of resveratrol are mediated through membrane-bound subpopulations of ERα and ERβ (Razandi et al. 1999, Evinger & Levin 2005). However, in this study, ER blockers, TAM and ICI, did not reverse the inhibitory effects of resveratrol on 11β-HSD1 (Fig. 3).

We evaluated the effect of resveratrol on 11β-HSD2 activity using rat kidney microsomes. Resveratrol did not inhibit 11β-HSD2 activity at concentrations below 10 μM (data not shown). At this level, resveratrol significantly inhibited 11β-HSD1 activity (Fig. 2C). These findings demonstrated that resveratrol was a selective inhibitor of 11β-HSD1. 11β-HSD2 converts an active glucocorticoid, cortisol or corticosterone, into its inactive form, cortisone or 11-dehydrocorticosterone respectively. The physiological role of 11β-HSD2 is to prevent mineralocorticoid receptors (MR) from being stimulated by cortisol or corticosterone and to cause MR to be activated by aldosterone in classical mineralocorticoid-responsive organs, the kidneys and colon (Mercer & Krozowski 1992, Albiston et al. 1994). Decreased 11β-HSD2 activity in the kidneys causes hypertension; therefore, selective inhibition of 11β-HSD1 without 11β-HSD2 inhibition is required for a therapeutic antiobesity agent.

In our previous study, the two 2-arylbenzofuran derivatives, 2-(4-hydroxyphenyl)-5-benzofuranol (Fig. 1C) and 2-(4-hydroxyphenyl)-6-benzofuranol (Fig. 1D), having a distance of ~12 Å between the two hydroxyl groups in their molecules, inhibited 11β-HSD1 activity without 11β-HSD2 inhibition (Kiyonaga et al. 2012). Therefore, two hydroxyl groups at the C-4’ and C-3 or C-5 positions with the same distance (~12 Å) in resveratrol may be crucial for the selective inhibition of 11β-HSD1. Resveratrol clearly exhibited features with selective inhibitory potency against 11β-HSD1, suggesting that resveratrol could be a seed compound for a novel class of selective inhibitors of 11β-HSD1. Further studies could be conducted to develop resveratrol analogs that are more active 11β-HSD1 inhibitors.

H6PD supplies NADPH, a critical cofactor for the full activation of 11β-HSD1 (Atanasov et al. 2004). Consistent with this finding, H6PD knockout mice lack 11β-HSD1 activity (Lavery et al. 2006). In this context, we investigated the effect of resveratrol on H6PD activity, and our results demonstrated that resveratrol did not inhibit H6PD in intact microsomes from rat mesenteric fat depots and liver (Fig. 4). These results indicate that the inhibitory effect of resveratrol on 11β-HSD1 activity may not be because of H6PD-mediated suppression of NADPH generation. In addition, these data showed that resveratrol does not inhibit G6PT, which is located in the membrane of the endoplasmic reticulum and supplies G6P to the lumen (Chou et al. 2002). Further, these results were consistent with features of previous studies, indicating that no inhibition of H6PD by E2 and 2-arylbenzofuran derivatives was observed (Tagawa et al. 2009, Kiyonaga et al. 2012). These characteristic properties might also be because of the typical structure of the spatial configuration of the two hydroxyl groups as observed in resveratrol, E2, or 2-arylbenzofuran derivatives.

The apparent $K_m$ for 11-dehydrocorticosterone of 11β-HSD1 in rat adipose tissue microsomes (0.66 μM; Fig. 6A) was similar to that of the liver preparation in this study. Those values approximated the values reported by others, which were 0.25 μM (McCormick et al. 2006) and 0.89 μM (Ge et al. 1997) in rats.

11β-HSD1 has been proven to be a potential therapeutic target for the treatment of central obesity and metabolic syndrome in experimental animals (Kotelevtsev et al. 1997, Masuzaki et al. 2001) and humans (Bujalska et al. 1997, Wake et al. 2003, Kannisto et al. 2004). It has been reported that classical 11β-HSD1 inhibitors, such as CBX and glycyrrhetinic acid, improved obesity, glucose tolerance, type 2 diabetes and metabolic syndrome, whereas these are also non-selective inhibitors (Andrews et al. 2003, Sandeep et al. 2005). More effective and selective 11β-HSD1 inhibitors have been developed in recent years. Accumulating evidence supports that the inhibition of 11β-HSD1 is a key strategy to treat metabolic syndrome. Considerable activity in the pharmaceutical industry has led to the discovery of several classes of 11β-HSD1 inhibitors (Boyle 2008, Anagnostis et al. 2013). Indeed, $K_i$ and IC50 values of resveratrol for 11β-HSD1 determined in this study were less effective than those of synthetic analogs developed as selective 11β-HSD1 inhibitors by pharmaceutical companies (Boyle 2008). However, resveratrol is a natural product, not a pharmaceutical agent. We have demonstrated that resveratrol, an ingredient of natural foods, such as grape skin, red wine, blueberries, and peanuts, which are easily and daily ingested, is an active 11β-HSD1 inhibitor. We also revealed one of the mechanisms of the effects caused by resveratrol. In this study, an inhibitory effect of 11β-HSD1 activity in microsomes of rat adipose tissue was observed at 1 μM resveratrol. According to the amount of resveratrol in the foods (Chachay et al. 2011), the appearance of 1 μM resveratrol in blood is equivalent to the ingestion of 13.7–27.4 g fresh grape skin or 130 ml red wine
(pinot noir). Therefore, it seems possible to ingest this amount of resveratrol daily. However, we would have to consider an individual’s coefficient of digestibility.

In conclusion, we demonstrated a non-competitive inhibitory effect of resveratrol on 11β-HSD1 in rodent adipocytes, suggesting a novel mechanism of regulating glucocorticoid metabolism.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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