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Blockade of the Renin-Angiotensin System Ameliorates Apelin Production in 3T3-L1 Adipocytes

Wei-Wen Hung · Tusty-Jiuan Hsieh · Tzu Lin · Pong-Chun Chou · Pi-Jung Hsiao · Kun-Der Lin · Shyi-Jang Shin

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Abstract
Purpose Angiotensin II (Ang II), the physiologically-active product of the renin-angiotensin system (RAS), has recently been found to be an adipokine secreted by adipocytes. Although Ang II is known to exert its effects via angiotensin II receptor type 1 (AT1R) or type 2 (AT2R), the roles of the two receptors in the adipose tissue are unclear. Apelin, another adipokine, has been found able to restore glucose tolerance in obese and insulin-resistant mice. Because they are both involved in metabolic disorders, there may be an interaction between the two adipokines.
Methods To observe the expression of RAS and apelin, 3T3-L1 adipocytes were harvested after 1, 2, 4, and 6 days of differentiation. The RAS blockers captopril (10^{-4} M), perindopril (10^{-3} M), losartan (10^{-4} M), or PD123319 (10^{-4} M) were added at day 2 of differentiation and harvested at day 4 and 6, when apelin expression was measured. Expressions of mRNAs were detected by real-time PCR. Production of Ang II and apelin was measured from culture media by ELISA. Cellular lipid droplets were detected by oil-red staining.
Results Our study showed that the mRNA expressions of AGT, renin, ACE1, and AT2R were up-regulated while AT1R mRNA was down-regulated during adipogenesis. Apelin expression increased during adipogenesis, and this increase was further augmented by blocking RAS. RAS blockers also prevented excessive lipid accumulation and the generation of ROS (reactive oxygen species) in differentiating adipocytes.
Conclusions Our study suggests that RAS blockers achieve their beneficial effects by their enhancement of adipocyte secretion of apelin.

Key words Adipocyte · Adipokine · Angiotensinogen · Angiotensin-converting enzyme inhibitor · Angiotensin receptor blocker

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Introduction

Obesity has become epidemic worldwide and its complications present a potential threat to life expectancy [1]. Metabolic dysfunction of adipose tissue might be the cause of several such complications such as diabetes and cardiovascular disease [2]. Adipose tissues regulate metabolic functions by secreting various adipokines [3]. Apelin, first isolated from adipose tissue in 2005, has been found to stimulate glucose utilization in normal and obese insulin-resistant mice [4, 5]. It is known that apelin mRNA levels increase during 3T3-L1 adipocyte differentiation [6], but

how apelin mRNA levels are regulated during this process remains unclear.

One of the physiologically-active adipokines that adipoctyes secret is Angiotensin II (Ang II), a downstream product of renin-angiotensin system (RAS) [7]. Systemic RAS is well-known to have angiotensinogen (AGT), which is produced within the liver, cleaved by renin from the kidney to become angiotensin I (Ang I), and then converted by angiotensin-converting enzyme-1 (ACE-1) to become angiotensin II (Ang II) [8]. Recently, however, RAS has been found not just work interdependently throughout the body, but also to exist and work independently in various organs including the brain, heart, kidney, and adipose tissues, etc [9]. Regulation by glucocorticoids to upregulate AGT gene expression and secretion in adpose cells has been confirmed and provided a potential link between obesity and hypertension [10].

In the cardiovascular system, Ang II induces vasoconstriction and cell proliferation or hypertrophy via angiotensin type I receptor (AT1R), while another angiotensin II receptor, AT2R, is thought to have opposite effect [9]. While the relationship between the two receptors may be clear in the cardiovascular system, their functions are not clearly understood in adipose tissue [11]. Although one study of rat and human adipocytes showed that Ang II increased lipogenesis via AT1R [12], another study showed Ang II exerted this effect via AT2R in 3T3-L1 adipocytes [13]. More recently, one study found that by knocking out AT2R in mice, adipose cell size could be reduced and that this gene might be manipulated to reverse obesity caused by the overexpression of angiotensinogen in the adipose tissue of mice [14]. Another study reported that by knocking down AT1R in mice, diet-induced weight gain and adiposity can be attenuated [15]. Moreover, use of AT1R blockers has been reported to reduce oxidative stress and ameliorate adipokine dysregulation in adipose tissue in obese mice [16] and to downsize adipocytes in diabetic mice [17].

In this study, we investigated whether or not RAS could regulate adipocyte secretion of apelin. To find out, we measured the mRNA expression and peptide production of apelin in 3T3-L1 adipocytes treated with various drugs known to block RAS: the ACE inhibitors, captopril (10^{-4} M) and perindopril (10^{-3} M); the AT1R blocker, losartan (10^{-4} M); or the AT2R blocker, PD123319 (10^{-4} M). We also studied the effect of this blockade on lipid accumulation and oxidative stress in these cells.

Materials and methods

Reagents and antibodies

D-glucose, 3-isobutyl-1-methylxanthine, dexamethasone, insulin, 10% formalin, oil red O, propylene glycol, nitro-blue tetrazolium (NBT), captopril and PD123319 were purchased from Sigma-Aldrich, Inc (Saint Louis, USA). Normal glucose (5 mM) DMEM (catalog no. 12320), penicillin/streptomycin, and fetal bovine serum (FBS) were bought from Invitrogen (Carlsbad, USA). AssayMax Human Angiotensin II ELISA Kit was purchased from Assaypro (St. Charles, USA). Apelin-12 EIA Kit was from Phoenix Pharmaceuticals, Inc (Burlingame, USA). Oligonucleotides were synthesized by Bio Basic Inc (Markham, Canada). Perindopril (S9780-1) was a gift from Les Laboratories SERVIER, France. Losartan potassium was a gift from MERCK Research Laboratories, Rahway, USA.

Study design

In order to observe the mRNA expression of various RAS components and adipokines, 3T3-L1 cells were harvested for RNA extraction at different timing after differentiation. The cell media were also collected for the detection of Ang II and apelin that were secreted from 3T3-L1 cells.

To observe the effect of RAS blockers on the expression of apelin mRNA, the 3T3-L1 adipocytes were cultured in DMEM supplemented with 25 mM glucose and 0.32 μM insulin with or without captopril (i.e. 10^{-4} M), perindopril (i.e. 10^{-3} M), losartan (i.e. 10^{-4} M), or PD123319 (i.e. 10^{-4} M) after 2 days of induction. The media and RAS blockers were changed every other day and the cells were harvested at day 4 and 6 for RNA extraction. Lipid accumulation in the adipoctyes was observed by oil red O staining and quantified by ELISA reader set at 490 nm. Oxidative stress was measured by NBT assay.

Cell culture

3T3-L1 preadipocytes (BCRC#60159; Bioresource Collection and Research Center, Taiwan) were cultured in DMEM with 10% FBS, 100 U/ml of penicillin, and 100 μg/ml of streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. 3T3-L1 preadipocytes were induced to differentiate by treatment with 25 mM D-glucose, 0.32 μM insulin, 0.5 mM 3-isobutyl-1-methylxanthine and 1 μM dexamethasone. After 2 days, the induction medium was removed and the cells were maintained in the culture medium with 25 mM D-glucose and 0.32 μM insulin. For each experiment, the same number of 3T3-L1 preadipocytes were seeded on 6-cm dishes and induced to differentiate after 2 days of reaching 100% confluence. The day of induction was defined as Day 0.

Oil red O staining

Lipid droplets were detected by oil red O staining [18]. 3T3-L1 cells were washed three times with PBS and fixed

in 10% formalin for 1 h. After being washed with PBS, the cells were stained with oil red O staining solution (0.5% oil red O in 100% propylene glycol). After staining, they were washed three times with water and observed under a phase contrast microscope. To quantify the lipid amount, stained cells were incubated with 100% isopropanol and shaken at room temperature for 20 min. The samples were read by ELISA reader set at 490 nm.

RNA extraction

Total RNA was extracted using Trizol reagent according to manufacturer's instructions (Invitrogen) and quantified by a Quant-iT™ RNA assay kit (Invitrogen).

Reverse transcription and real time PCR

Two μg of total RNA was used to synthesize first-strand cDNAs with a SuperScript™ III reversed transcriptase kit following manufacturer's instructions (Invitrogen). Then, the first-strand cDNA was diluted with water to a ratio of 1:9, and aliquots were processed to amplify the cDNA fragments with the iQ™ SYBR Green Supermix according to manufacturer's directions (Bio-Red Laboratories, Hercules, USA). First-strand cDNA and primers were added to a final volume of 25 μl PCR mixture. The PCR mixtures were amplified in a MiniOpticon real time PCR system (Bio-Red Laboratories, Hercules, USA). Primers were designed by Beacon Designer 4 software (Premier Biosoft Int., Palo Alto, USA). The sequences are shown in Table 1.

ELISA

3T3-L1 cells were differentiated and treated with RAS blockers as described previously. Then, the conditioned media from each sample were collected. The concentration of Ang II and apelin in each sample was determined by using an AssayMax Human Angiotensin II ELISA Kit and an apelin-12 EIA Kit, respectively.

NBT essay

The nitroblue tetrazolium (NBT) assay is a laboratory technique used to evaluate ROS production [19]. NBT will be reduced by ROS to a dark-blue insoluble form of NBT called formazan. After washing with PBS, 3T3-L1 adipocytes were incubated in PBS containing 0.2% NBT. After 90 min, formazan was dissolved in 50% acetic acid and the absorbance was determined at 560 nm.

Statistical analysis

At least four separate independent experiments were performed per procedure, and each treatment group was run in duplicate for real time PCR reactions and ELISA. The data were analyzed by unpaired Student's t-test, one-way analysis of variance, and the Bonferroni test on GraphPad Prism software. All data are presented as means ± SE and a probability level of $P<0.05$ was regarded as significant.

Results

To observe the mRNA expression pattern of the related components in renin-angiotensin system during the process of adipogenesis, 3T3-L1 preadipocytes were induced to differentiate and harvested on day 1, 2, 4, and 6. As can be seen in Fig. 1a, the AGT mRNA was over-expressed beginning on day 1 after induction and increased gradually to more than 100-fold over the 6-day adipogenesis process. Figure 1b and d show that on day 2 the expression of ACE1 and AT2R mRNA peaked with 40-fold and 15-fold increases, respectively. Figure 1c shows that AT1R mRNA

Table 1 Primer sequences

Gene	Sense (5′-3′)	Antisense (5′-3′)	Gene number
AGT	aagcaggagaggaggaacagc	cgtagatggcgaacaggaagg	NM_007428
Renin	cgacccgcagcattatcaagg	tccaccactaccgcacagc	NM_031192
ACE1	ggcagtggctacgagtatgac	atagttctccttggtgatgcttcc	NM_009598
AT1R	caagtcgcactcaagcctgtc	tcactccacctcagaacaagacg	NM_177322
AT2R	ggtctgctgggattgccttaatg	acttggtcacgggtaattctgttc	NM_007429
Apelin	ctttcccaaagccttcctatc	aagaggtgaggacgctatgc	NM_013912
Adiponectin	ggagagaaaggagatgcaggt	ctttcctgccaggggttc	NM_009605
RBP4	gacacggaggctggtgag	ggcctgctttgacagtaacc	NM_011255
Resistin	cttcaactccctgtttccaaatgc	gctcaagactgctgtgccttc	NM_022984
Visfatin	gtgcctgtgtctgtggtc	tgatgattagtggtgcctctg	NM_021524
TNF-α	cctcttctcattcctgcttgtgg	gtttgctacgacgtgggctac	NM_013693
β-actin	gaaatcgtgcgtgacatc	ccatacccaagaaggaagg	NM_007393

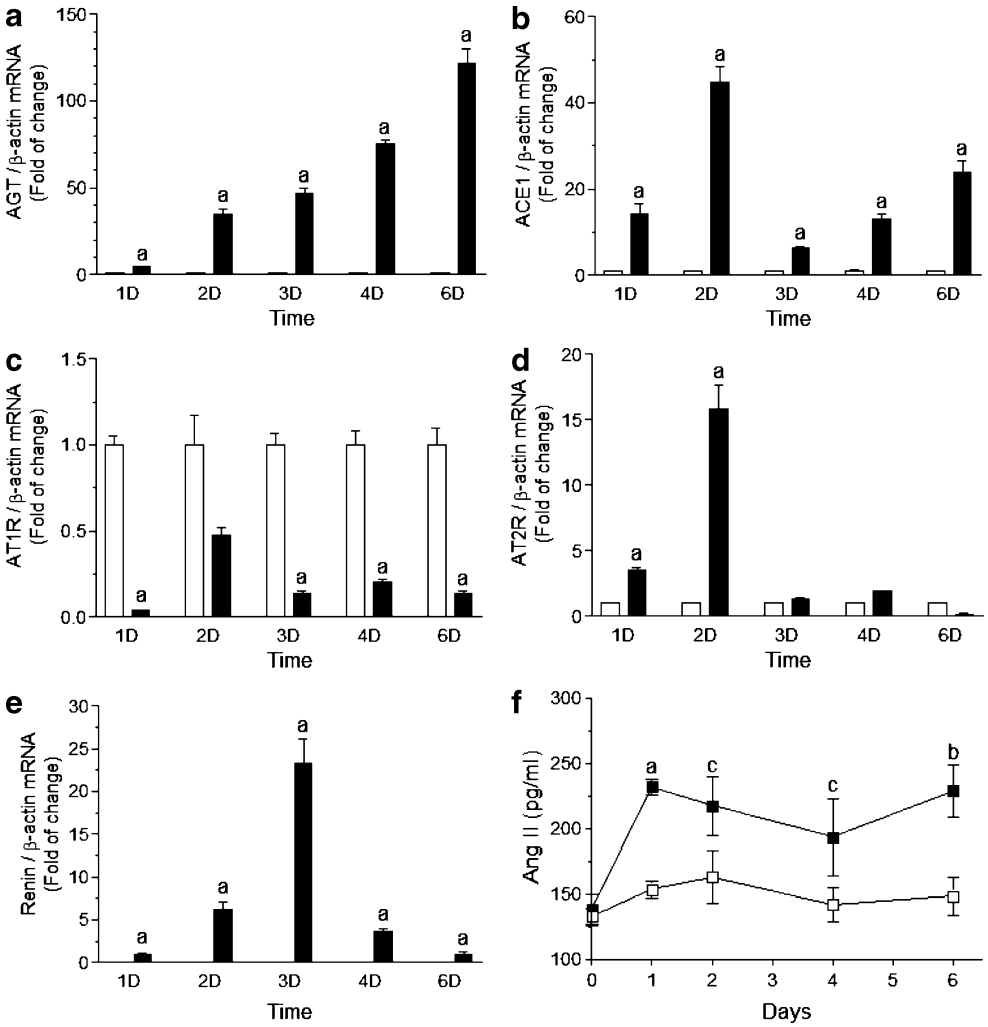

Fig. 1 Expressions of RAS components during adipogenesis in 3T3-L1 cells. The mRNA expressions of angiotensinogen (AGT; **a**), Angiotensin-converting enzyme-1 (ACE1; **b**), Ang II receptor type 1 (AT1R; **c**), Ang II receptor type 2 (AT2R; **d**) and renin (**e**) were detected by real-time PCR. The Ang II in culture medium was measured by an ELISA kit. (□) control; (■) induction. Data are the means ± SD. (a: $P<0.001$, b: $P<0.01$, c: $P<0.05$, induction vs. control of the same day)

expression remained low from day 1 to day 6 compared to the controls. The level of renin mRNA was undetectable in preadipocytes after a 45-cycle real-time PCR reaction (Fig. 1e); however, the renin mRNA expression in adipocytes was first detected at about 35-cycle by real-time PCR on day 1 after induction and increased more than 20 folds on day 3 compared to day 1 (Fig. 1e). We further measured the production of Ang II that was secreted into the 3T3-L1 cell medium by ELISA (Fig. 1f). The Ang II production began to increase on day 1, and the increase continued throughout the 6-day course.

We also monitored the mRNA expressions of apelin during adipogenesis. As is shown in Fig. 2a, the expression of apelin mRNA increased gradually during adipogenesis, reaching a more than 20-fold increase by day 6. Figure 2b shows that there were significant increases in apelin peptide production on day 4 and day 6. In order to know the expressive pattern of other adipokines, we detected the mRNA levels of adiponectin, retinol binding protein 4 (RBP4), resistin, and visfatin. As Fig. 3 shown, mRNA expressions of these four adipokines were greatly upregulated in fully differentiated 3T3-L1 adipocytes.

We used ACE inhibitors (ACEI; captopril and perindopril), AT1 receptor blocker (losartan), and AT2 receptor blocker (PD123319) to block the effect of endogenous Ang II to study the interactions between each component of the renin-angiotensin system and changes in apelin in adipocytes. Each drug was added to the cell medium after 2 days of differentiation and harvested on day 4 and day 6 to measure the changes of apelin mRNA expression and peptide production. Figure 4a shows that on day 4 the expression of apelin mRNA was three times higher in the induction group than in the controls and that these increases could be further augmented with the addition of either captopril, perindopril, losartan, or PD123319. As can be seen in Fig. 4b, like the increases in apelin mRNA expression, we also found a significant increase in the apelin peptide that was secreted into the cell medium of the induction group on day 6. The addition of captopril, perindopril, losartan, or PD123319 also significantly enhanced the secretion of apelin in these adipocytes.

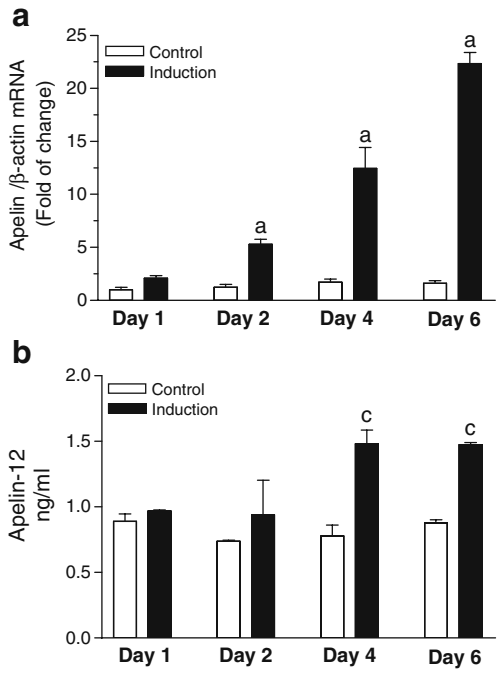

Fig. 2 Expression of apelin during adipogenesis in 3T3-L1 cells. **a** The expression of apelin mRNA. **b** The apelin released from 3T3-L1 cells into culture medium was detected by an ELISA kit. Data are the means ± SD. (*a*: $P<0.001$ and *c*: $P<0.05$ v.s. induction day 1)

To compare the effects of RAS blockers on apelin with the inflammatory adipokines, we detected the mRNA levels of RBP4, resistin, and visfatin. In contrast to the expression of apelin, RAS blockers reduced the mRNA expressions of RBP4 (Fig. 5a), resistin (Fig. 5b), and visfatin (Fig. 5c) in 3T3-L1 adipocytes. We also attempted to compare the influence of RAS blockers on TNF-α expression, but we observed that the mRNA level increased only on day 2 after differentiation in 3T3-L1 adipocytes (Fig. 5d).

We wanted to evaluate whether the blockade of RAS would affect the lipid formation in adipocytes. To do this, we used oil-red O staining to observe lipid accumulation in the adipocytes which we quantified using ELISA reader. Figure 6b shows the mature adipocytes in the induction group. The differentiating adipocytes treated with captopril (Fig. 6c), perindopril (Fig. 6d), losartan (Fig. 6e), or PD123319 (Fig. 6f) were found to have less cellular lipid accumulation than the untreated adipocytes. Figure 6g, a summary of our ELISA reader results, shows that the differences were significant (all $p<0.001$). To study the effect of this blockade on oxidative stress, we measured the reactive oxygen species (ROS) generated by the differentiated adipocytes using nitroblue tetrazolium (NBT) assay (Fig. 6h). We found that the generation of ROS increased simultaneously as lipids accumulated in differentiating adipocytes. Treatment with either captopril, perindopril, losartan, or PD123319 significantly suppressed the generation of ROS in these cells (all $p<0.01$).

Discussion

In this study designed to clarify the functions of the two Ang II receptors during adipocyte differentiation, we analyzed the mRNA changes of the two receptors. We found the mRNA expression of AT1R to be down-regulated

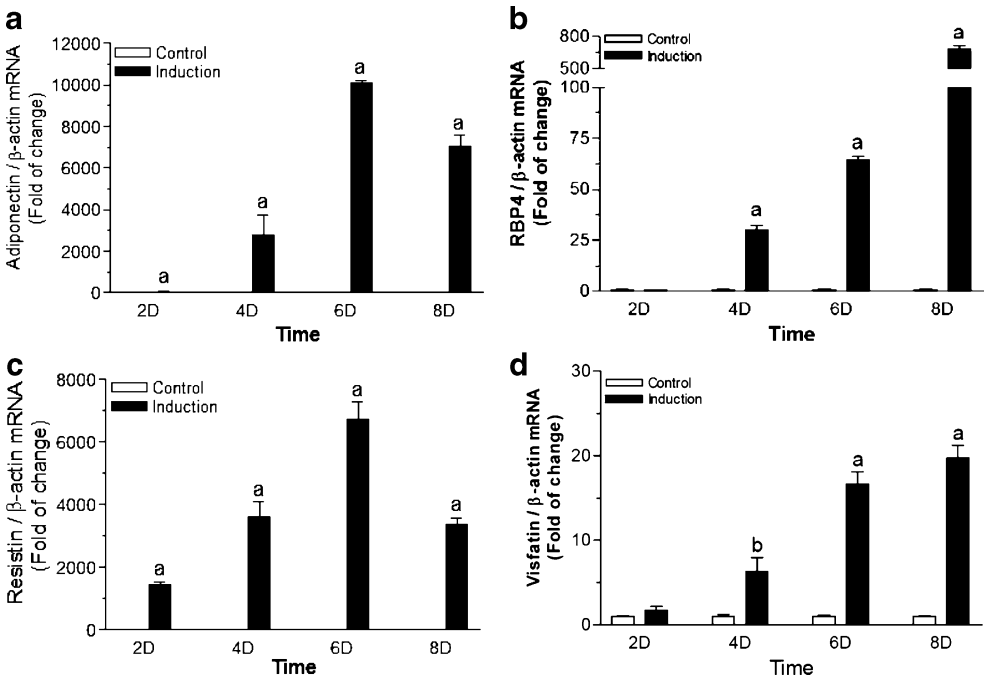

Fig. 3 Messenger RNA expressions of **a** adiponectin, **b** RBP4, **c** resistin , and **d** visfatin. 3T3-L1 cells were harvested on day 2, 4, 6, and 8 days of differentiation for RNA extraction and mRNA expressions were detected by real-time PCR. Data are the means ± SD. (*a*: $P<0.001$, *b*: $P<0.01$, induction vs. control of the same day)

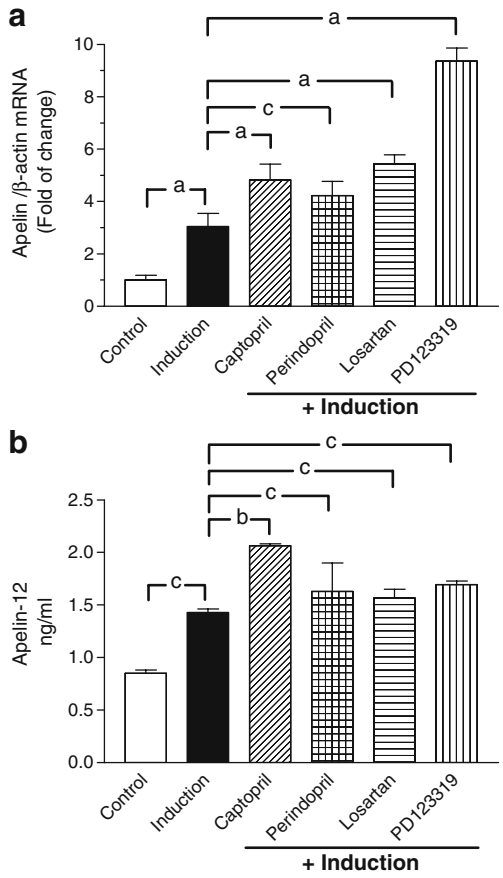

Fig. 4 Effects of RAS blockers on apelin expression in 3T3-L1 adipocytes. **a** Apelin mRNA expression on day 4 after differentiation. **b** Apelin peptide released from 3T3-L1 cells on day 6 after differentiation. Captopril (10^{-4} M), perindopril (10^{-3} M), losartan (10^{-4} M) and PD123319 (10^{-4} M) were added into culture medium on day 2 after differentiation. Data are the means ± SD. (*a*: $P<0.001$, *b*: $P<0.01$, and *c*: $P<0.05$ v.s. induction)

and that of AT2R to be up-regulated during the 6-day adipogenesis process. AT2R peaked at day 2. Blocking these receptors resulted in increased mRNA expression and peptide production of apelin throughout the 6-day adipogenesis process. Taken together, our results show that apelin can be increased by blocking RAS during adipocyte differentiation.

Local renin-angiotensin system was found to exist locally in adipose tissue in 1987 when angiotensinogen (AGT) mRNA was found in rat periaortal adipose tissue [20]. Study further confirmed that AGT mRNA increased in differentiating adipocytes [21]. Since that time, mRNA and proteins of the Ang II-forming enzymes in the RAS, including renin and ACE1, have been identified in various rodent or human adipocyte studies [22, 23], while Ang II was shown to have effects on human adipose conversion and gene expression of RAS [24]. Jones et al. showed that Ang II increased lipogenesis through AT2R in 3T3-L1 adipocytes [13], while another study by Mallow et al. detected AT1 receptor proteins throughout the 12-day developmental period of 3T3-L1 cells [25]. Other in vivo studies in mice also reported that AT1R was the predominant Ang II receptor during adipogenesis [15, 17], while others found AT2R to predominate [14, 26]. In our study, the mRNA expression of AT1R mRNA was down-regulated (Fig. 1c) and AT2R mRNA was upregulated (Fig. 1d) during adipocyte differentiation. These findings were consistent with those of Jones et al [13] but not Mallow et al [25]. The apparent difference might be due to the length of experimental period. Mallow et al [25] conducted 3T3-L1 preadipocyte cultures up to 12 days. In their experiments, AT2R proteins could also be detected on day 6, compatible with our experiments. We speculate that AT2R might play an important role in lipid accumulation at an earlier stage of adipogenesis and AT1R at a later stage of adipocyte differentiation. Renin mRNA expression in preadipocytes was undetectable in our experiment. In contrast, a trace amount of renin mRNA expression was first detected on the day one after induction of adipocyte differentiation and the mRNA level was increased more than 20 folds on the day three compared to day one. Our result was in agreement with the previous study of Fowler et al [27]. However, we observed that the Ang II peptide was significantly increased in the culture medium since day one, indicating that other factors may be involved in the production of Ang II in adipocytes. It has been postulated that other enzymes which form angiotensin I from angiotensinogen might participate in the step, including cathepsin D, pepsin, and renin-like enzymes [28].

Our study found the adipokine apelin to increase during the differentiation of 3T3-L1 adipocytes. These results are consistent with the findings of a previous investigation that demonstrated that apelin mRNA gradually increased along with increases in triglyceride content during 3T3-L1 adipocyte differentiation, reaching a maximum 4 days after the induction of differentiation and gradually decreasing after day 6 [6]. We further analyzed the peptide production of apelin, which we found to increase along with increases in mRNA levels during the 6-day adipogenesis process. The addition of the different RAS blockers (captopril, perindopril, losartan, or PD123319) further increased the mRNA expression and the peptide production of apelin. To the best of our knowledge, this study is the first to show that RAS blockers can enhance apelin expressions in adipocytes. These findings show an association between RAS and apelin during adipogenesis. According to our results, the mRNA levels of adipokines such as adiponectin, resistin, RBP4 and visfatin were upregulated along with the increase of cell size and cellular lipid amount of adipocytes. Studies have demonstrated that RAS blockers decrease cell size and lipogenesis in adipocytes [29, 30]; hence, it could be speculated that RAS blockers may alter the expression of

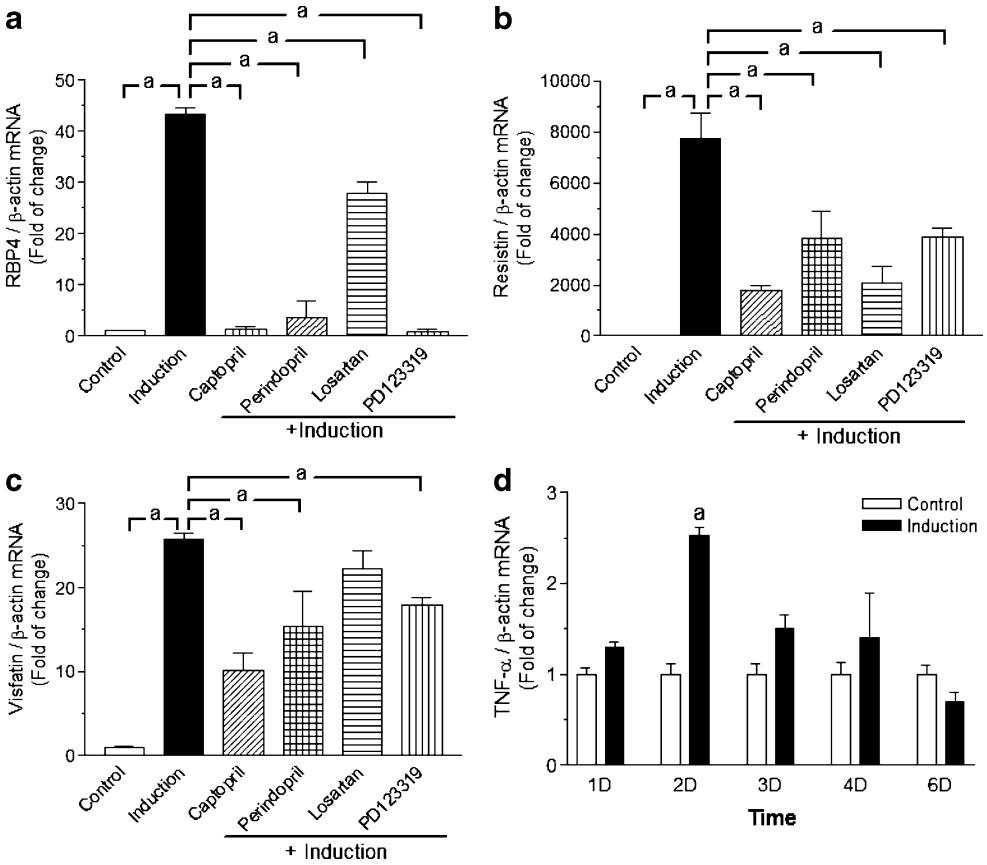

Fig. 5 Effects of RAS blockers on **a** RBP4, **b** resistin, and **c** visfatin mRNA expressions in 3T3-L1 adipocytes. Captopril (10^{-4} M), perindopril (10^{-3} M), losartan (10^{-4} M) and PD123319 (10^{-4} M) were added into culture medium on day 2 after differentiation. Data are the means ± SD. (*a*: $P<0.001$) (D) TNF-α mRNA expressions on day 1, 2, 3, 4, and 6 after differentiation. (*a*: $P<0.001$, induction vs. control of the same day)

some adipokines. Our results showed that the mRNA expressions of resistin, RBP4 and visfatin were down-regulated after the treatment of RAS blockers. This observation was in contrast with the result that RAS blockers increased the expression of apelin, indicating that the upregulation of apelin was an independent event from the regulation of other adipokines by the treatment of RAS blockers.

Human apelin and its receptor APJ have been found in various organs, including brain, heart, lung, liver, and kidney [31], and they have been reported to be related to the modulation of food intake [32], the increase in positive inotropic effects [33], the development of liver cirrhosis [34], maintenance of insulin sensitivity [35], and regulation of body adiposity [36]. In a mouse model of atherosclerosis, treatment with apelin has been shown to block the effects of Ang II [37]. However, the role of apelin in obesity is still unclear. To our knowledge, there is no report indicating that apelin directly regulates adipogenesis in adipocytes. Reports that address the relationship between adipogenesis and apelin production were based on the observation that the increase of apelin is accompanied with obesity or the differentiation of adipocytes [4, 6]. Therefore, a further investigation is needed to clarify the role of apelin in adipogenesis. In clinical studies, apelin plasma levels in obese patients were found to be significantly higher than control lean individuals [38] while another study showed that weight loss by hypocaloric diet in obese women could reduce plasma apelin levels [39]. Li et al. reported increased levels of apelin, while Erdem et al. reported low plasma apelin levels in type 2 diabetic patients [40, 41]. Erdem et al. [41] concluded the difference between their study and that of Li et al could have resulted from differences in the populations enrolled for each study. Li et al [40] recruited diabetic patients under treatment while Erdem et al [41] included newly diagnosed and untreated type 2 diabetes mellitus patients with no additional disorders related to inflammation or no use of any medication. Besides, Erdem et al., finding a positive correlation between plasma apelin and adiponectin, concluded that the mechanism for the regulation of apelin and adiponectin seemed to be similar [41]. One recent study has shown that losartan can enhance the production of adiponectin in 3T3-L1 adipocytes [42]. Similarly, we found that the addition of losartan augmented the production of apelin, leading us to conclude that RAS might be involved in the regulation of both adiponectin and apelin. Adiponectin is a protective adipokine that has anti-inflammatory and anti-atherosclerotic effects, and a deficient of adiponectin has been associated with obesity-related disorders such as diabetes and cardiovascular disease [43]. Apelin is classified as another protective adipokine and is suggested

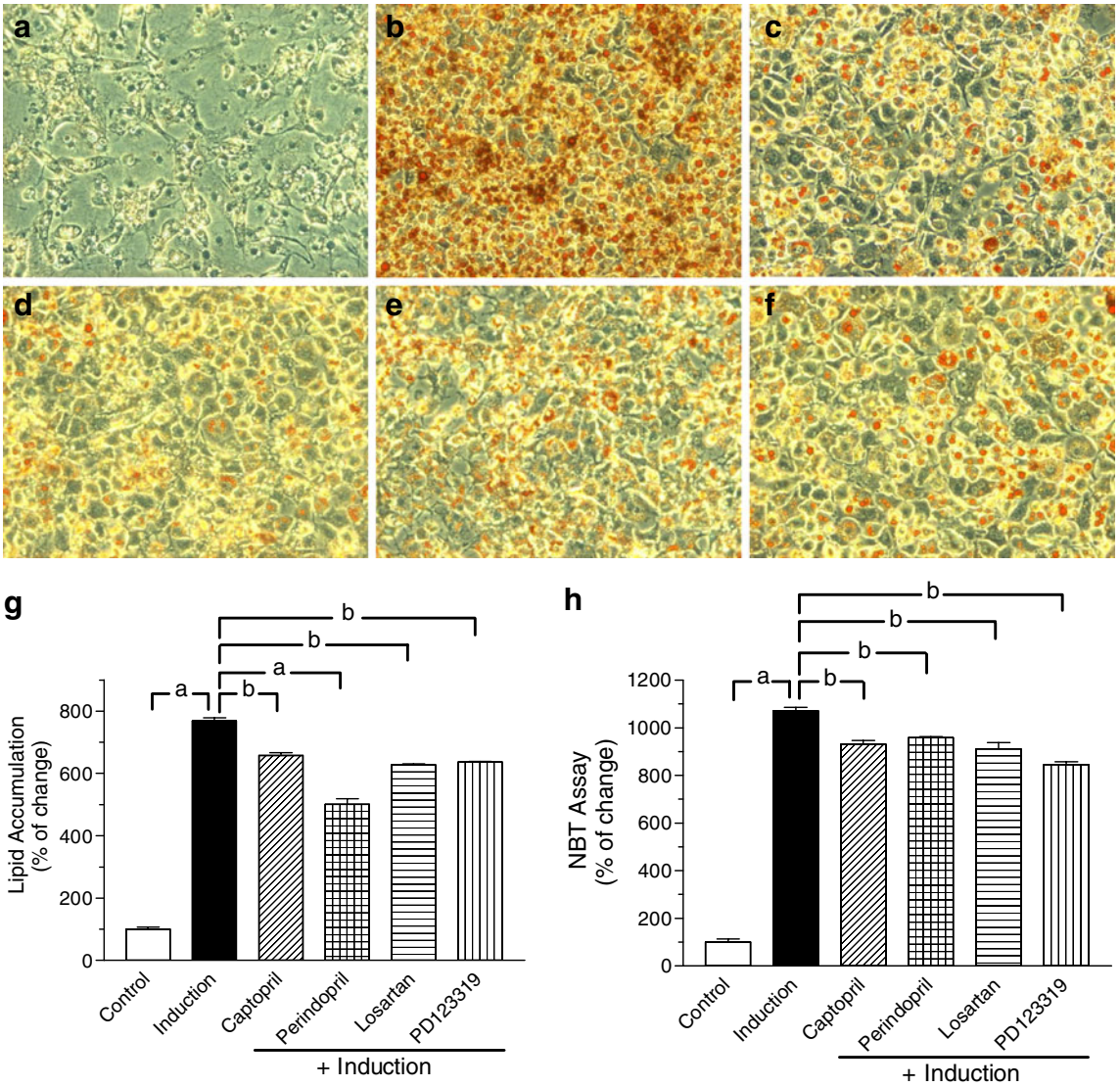

Fig. 6 Effects of RAS blockers on lipid accumulations and oxidative stress. Cellular lipid was stained by Oil Red. **a** Control; **b** differentiated adipocytes. Captopril 10^{-4} M (**c**), perindopril 10^{-3} M (**d**), losartan 10^{-4} M (**e**), and PD123319 10^{-4} M (**f**) decreased lipid accumulations in differentiated adipocytes. **g** Cellular lipid was quantified by dissolving the Oil Red in isopropanol and then measured by an ELISA reader. **h** The production of reactive oxygen species was quantified by a nitroblue tetrazolium (*NBT*) assay. Data are the means ± SD. (*a*: $P<0.001$, *b*: $P<0.01$, and *c*: $P<0.05$ vs. induction)

to have antiatherogenic properties [44–46]. Apelin infusion showed to significantly reduce aortic macrophage colony-stimulating factor expression and decreased monocyte chemattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1alpha, interleukin (IL)-6, and tumor necrosis factor (TNF)-α mRNA levels [46]. According to our result, the mRNA level of TNF-α was increased only on the day two after differentiation and our result was in agreement with that of Wang et al. [47]. In our experimental model, the RAS blockers were added in to the cell medium on the day two after differentiation; therefore, we were not able to observe whether or not the expression of TNF-α was inhibited by RAS blockers. However, we observed that the RAS blockers inhibited the mRNA overexpressions of adipokines related to inflammation, such as RBP4, resistin, and visfatin [44, 48]. Promotion of apelin production from adipocytes by RAS blockers may counteract the harmful effects of inflammatory cytokines that produced by the adipocytes with excessive lipid accumulation. Our finding suggests that the pharmacological mechanisms of RAS blockers may be, at least in part, via the increase in protective adipokine apelin and the reduction of inflammatory adipokines such as RBP4, resistin, and visfatin.

This study found that either losartan (AT1R blocker) or PD123319 (AT2R blocker) could inhibit lipid accumulation (Fig. 4) and increase apelin mRNA expression and peptide

production (Fig. 3) in adipocytes. Although the two findings appear to contradict each other, in vivo studies of gene knock-out mice have found that a deficiency in AT1R or AT2R deficiency can protect these mice from high-fat diet-induced obesity [15, 26]. Both receptors may contribute to the adiposity induced by Ang II, with Ang II acting as an antilipolytic hormone via AT1R or a lipogenic hormone via AT2R in adipocytes [14]. There have also been studies of obese mice [16] and diabetic mice [17, 49] that show AT1R blockers can reduce the size of adipocytes. Our study, however, found a down regulation in mRNA of AT1R from day 1 to day 6 after the induction of differentiation, making it difficult to explain the reason that AT1R blockers can effectively reduce lipid accumulation in 3T3-L1 adipocytes. We speculate that the use of AT1R blockers might somehow interfere with the deleterious actions of Ang II on adipocytes. Further study is needed to understand what mechanism might be involved in such an interference.

Conclusion

In conclusion, our study suggests that Ang II acts via AT2R at an earlier stage of 3T3-L1 adipocyte differentiation to induce lipid accumulation. Ang II induces adipocytes to produce the adipokine, apelin, thereby reducing oxidative stress during adipogenesis. ACEI, AT1R blocker, and AT2R blocker not only reduce the deleterious effects of Ang II on adipocytes but also increase the production of apelin through which they achieve their protective effect.

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