We have shown previously that stimulation of the angiotensin II type 2 receptor (AT2R) results in nerve facilitation. In this study, we determined the capacity of candesartan to correct expression patterns characteristic of neuropathy and AT2R-mediated neurite outgrowth in the fructose-induced insulin-resistant rat, which is one of the human hyperinsulinemia models. Wistar rats received a 15% (w/v) fructose solution in their drinking water for 4 weeks (fructose-drinking rats [FDRs]), with or without candesartan (5 mg/kg/day). We evaluated physiological and behavioral parameters and performed immunohistochemical studies. We found that the FDR developed insulin resistance and downregulated both AT2R neuronal function and phosphorylated Akt expression in dorsal root ganglia (DRG) neurons. Candesartan improved neurite outgrowth in the FDR, which was associated with the restoration of AT2R and phosphorylated Akt expression. Furthermore, downregulation of phosphoinositide 3-kinase (PI3K) inhibited AT2R-mediated neurite outgrowth in control DRG cells. PI3K activation increased AT2R-mediated neurite outgrowth and phosphorylated Akt expression in FDR DRG cells. These results suggest that the decrease of AT2R-mediated neurite outgrowth in FDRs is likely to be the result of decreased PI3K-dependent Akt activation. Candesartan improved AT2R neuronal function and Akt phosphorylation, which were associated with sensory nerve defects and insulin sensitivity in the FDR.

Angiotensin II (Ang II) has been shown to be involved in the development of insulin resistance. Current evidence indicates that Ang II can negatively modulate the muscle insulin signaling pathway, leading to reduced insulin-stimulated glucose uptake (1,2), GLUT4 translocation (3), and inhibition of the insulin signaling pathway through Ang II type 1 receptors (AT1Rs) (4). Ang II activates two major types of seven-transmembrane domain G protein–coupled receptors: the AT1R and Ang II type 2 receptors (AT2Rs). AT1R function as regulators of the cardiovascular system, oxidative stress, and cell proliferation, whereas AT2Rs mediate the opposite effect of AT1Rs such as vasodilatation. AT2R mRNA is highly expressed in neonates but decreases after birth (5). However, in pathological conditions such as vascular injury or atherosclerosis, the expression of AT2Rs is significantly increased (6). The AT2R is known to mediate the effects of nerve regeneration. Many reports have demonstrated that AT2Rs are involved in neuronal differentiation of PC12 cells (7).

Diabetic neuropathy is known to be associated with hyperglycemia. We demonstrated previously that fructose-drinking rats (FDR) mimic human metabolic syndrome, develop hyperinsulinemia but not hyperglycemia, and suppress the function and innervation of calcitonin gene–related peptide (CGRP), which is a major neurotransmitter in sensory nerves and is produced in the dorsal root ganglia (DRG) (8,9). Moreover, previous studies in our laboratory have shown that activation of AT2Rs facilitates reinnervation of mesenteric peripheral CGRP-containing nerves in nerve-injured rats after phenol treatment (10). Given the neurite outgrowth function of AT2Rs, we hypothesized that in insulin-resistant rats, which have impaired peripheral nerve function, neurite outgrowth may be affected via the AT2R. The purpose of the current study was to ascertain whether depressed CGRP nerves after fructose-induced insulin resistance was related to AT2R inactivation and to determine whether treatment with the AT2R antagonist candesartan cilexetil improves this depressed response. In addition, we focused on the phosphoinositide 3-kinase (PI3K)-Akt signaling pathway. A study has shown that Akt phosphorylation has consistently shown to have a positive influence on neurite elongation (11). Therefore, we investigated whether insulin resistance affected AT2R-mediated neurite outgrowth, particularly focusing on the PI3K-Akt pathway.

**RESEARCH DESIGN AND METHODS**

All animal procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the Japanese association for laboratory animal science. All experiments were approved by the Animal Care and Use Committee of the Okayama University of Science. According to these guidelines, efforts were made to minimize the number of animals used and their discomfort.

**Fructose-drinking rats.** According to a method described previously (8), 25 6-week-old Wistar rats (purchased from Shimizu Experimental Animals, Shizuoka, Japan) were given normal drinking water (control group), and 50 were given 15% (w/v) fructose solution (fructose group). The 15% (w/v) fructose solution was given as drinking water from 6 weeks of age to 10 weeks of age. For drug treatment, animals received a daily oral administration of candesartan cilexetil (5 mg/kg/day; Takeda Pharmaceutical, Japan, Okayama) with 15% (w/v) fructose solution.

**Oral glucose tolerance test.** At 10 weeks of age, rats were fasted overnight and then given an oral administration of 4g glucose (2 g/kg body weight dissolved in 0.9% [w/v] saline). At 0, 30, 120, and 180 min after glucose administration, blood samples were taken from the caudate vein. The plasma level of glucose and insulin were measured using a glucose analyzer (ACCU-CHEK Advantage glucose Testing System; Roche Diagnostics, Indianapolis, IN) and an ELISA insulin kit (Morinaga Biochemistry, Kanagawa, Japan), respectively. This ultrasensitive rat sandwich ELISA kit is designed for detection of insulin (0.1–6.4 ng/mL) in plasma, serum, and cell culture supernatant.
Hot-plate test. An animal was placed on an aluminum plate (45 × 30 cm) maintained at 55 ± 0.5°C. Rats were placed on the hot plate, and the time taken to either lick the fore or hind-paws or jump off the hot plate was recorded as the latency time. The latency to respond was measured using a stopwatch. A cutoff time (15 s) was set to avoid tissue damage.

Tail immersion test. An animal was gently restrained, and the distal half of the tail was immersed in a water bath at 50°C and the withdrawal latency for tail flicking was recorded using a stopwatch. A cutoff time (15 s) was set to avoid tissue damage.

Immunohistochemistry. Immunohistochemical studies were evaluated as previously reported (4, 5). In brief, animals were treated with a large dose of sodium pentobarbital (50 mg/kg i.p.). The superior mesenteric artery was removed and immersion-fixed in Zamboni solution for 48 h. After fixation, the artery was repeatedly washed in 10 mM PBS (pH 7.4) and immersed in PBS containing 0.5% (v/v) Triton X-100 overnight, and incubated with PBS containing normal goat serum (1:100) for 60 min. The tissue was then incubated with rabbit polyclonal anti-CGRP (Enzo Life Sciences, Farmingdale, NY) at a dilution of 1:1,000 for 15 h. After being washed, the artery was washed in PBS and the sites of antigen-antibody reaction were detected by incubation with fluorescein-5-isothiocyanate (FITC)-labeled goat-anti-rabbit IgG (diluted 1:1,000) (ICN Pharmaceuticals, Aurora, OH) for 60 min. Thereafter, the artery was observed under a confocal laser scanning microscope (CLSM510; Carl Zeiss, Tokyo, Japan) in Okayama University Medical School Central Research Laboratory.

Immunohistochemical analysis. The immunostaining density of CGRP-like immunoreactive (CGRP-LI) nerve fibers was analyzed using a method described previously (10). All counts and measurements were performed by an investigator blinded to the source material, including the treatment of rats. For quantitative evaluation of CGRP-LI, confocal projection images of CGRP-immunostained, which consisted of 8–10 overlapping images (0.1 μm scanning) patched together, were magnified 20× and digitized as TIFF images using a digital camera system (Olympus SP-1000; Olympus, Tokyo, Japan) and imported into a Windows XP computer (Toshiba, Tokyo, Japan). The stored digital images were analyzed using image-processing software (Simple PCI; Compix Imaging Systems, Cranberry Township, PA). The extraction of specific color and measured field commands were used to extract the CGRP-LI areas (which were stained green). Extraction of the signal was performed out using specific protocols based on the hue, lightness, and saturation color parameters. A measured color was recorded as the latency time. The latency to respond was measured using a chemiluminescent substrate kit (GE Healthcare). Bands were analyzed by densitometry using FluorchemTM8800 (α-Innotech, San Leandro, CA), and the content of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which was detected using a rabbit anti-GAPDH antibody (1:10,000; Sigma), was used as a control to ensure that the same amount of protein was loaded in each lane.

Statistical analysis. All data are expressed as the mean ± SEM. Comparisons between two values were analyzed using the Student t test. Analysis of variance followed by Tukey’s test was used to determine statistical significance where appropriate. A P value <0.05 was considered statistically significant.

RESULTS

Plasma glucose and insulin levels. There was no significant difference in body weight and fasting blood glucose levels between the control and FDR groups (Table 1). However, plasma insulin levels were significantly higher in the FDR group when compared with the control (Table 1; P = 0.0060). The homeostasis model assessment insulin resistance also significantly increased in the FDR group (Table 1; P = 0.0319). These data indicate that fructose treatment induced insulin resistance as described previously (8, 9). Blood glucose levels during the oral glucose tolerance test are shown in Fig. 1A. After the administration of glucose at 120 min, blood glucose levels in the FDR group were significantly higher than the control or FDR + CAN group (AT1R antagonist candesartan cilexetil) group. Plasma insulin levels were also much higher in the FDR group when compared with the control and FDR + CAN group (Fig. 1B). These results indicate that the FDR group had hyperinsulinemia and that AT1R blockade induced the alleviation of hyperglycemia and hyperinsulinemia.

Effect of AT1R blockade on thermal nociception and innervation of CGRP-LI fibers in mesenteric arteries. During the hot-plate test, licking or jumping responses were considered to be a result of supraspinal sensory integration (12). To test sensory performance at the

| TABLE 1 | Physiological parameters determined in rats after administration of water (control) or 15% (w/v) fructose (FDR) for 4 weeks |
|---------|-------------------------------------------------------------------------------------------------------------------------------------|
| n       | 7                                                                                                                                     |
| Glucose (mg/dL) | 109 ± 3.8                                                                  | 109 ± 4.7                                                                    |
| Insulin (mIU/L) | 26.9 ± 1.5                                                                  | 35.1 ± 2.3*                                                                  |
| HOMA-IR   | 7.26 ± 0.5                                                                   | 9.46 ± 0.9*                                                                  |
| Body weight | 295 ± 7.8                                                                    | 305 ± 8.7                                                                    |

Values are expressed as the mean ± SEM. HOMA-IR, homeostasis model assessment insulin resistance. *P < 0.05 vs. control.
supraspinal level, we measured the latency to respond when the hot plate was set at 55°C (Fig. 2A). The cutoff time was set at 30 s. Furthermore, the tail immersion test was performed for the assessment of thermal nociception (Fig. 2B). A significant increase in latency was observed in the FDR group compared with control rats in both tests, indicating that the FDR group had reduced sensitivity to noxious thermal stimuli. Administration of the AT1R antagonist candesartan cilexetil improved the response to thermal nociception in the FDR group.

Typical patterns of CGRP-LI nerve fibers in the small mesenteric arteries of control rats and the FDR group were observed. In addition, the distal mesenteric artery was densely innervated by CGRP-LI nerve fibers (Fig. 2C, top). The density of CGRP-LI nerve fibers in the FDR group was markedly decreased by ~20% compared with age-matched control rats. In contrast, administration of the AT1R antagonist candesartan cilexetil improved the response to thermal nociception in the FDR group.

Effect of AT1R blockade on AT2R expression and function in DRG cells. We next questioned why AT1R blockade improved peripheral nerve disorder in the FDR group. For this, we focused on AT2R expression and function in the DRG isolated from control rats, the FDR group, and FDR + CAN. According to our previous report (10), AT2R stimulation facilitated reinnervation of mesenteric perivascular CGRP containing nerves in rats. AT2Rs were detected as membrane proteins with a molecular weight of 47 kDa (Fig. 3A, top). In the FDR group, AT2R expression was significantly higher than control rats. Candesartan cilexetil administration led to the suppression of high AT2R expression (Fig. 3A, bottom). To determine the function of AT2Rs in the DRG, we examined whether AT2R activation could affect the neuronal sprouting response in DRG cells from the control, FDR, and FDR + CAN groups. Immunostaining showed CGRP-LI–positive neurons and neurite outgrowth (Fig. 3B, top). To activate the AT2R, we used 10 nmol/L CGP42112, which is a selective agonist of AT2Rs. The representative images showed different neurite growth patterns after 4 days of treatment with CGP42112. In the DRG of the FDR group, AT2R activation did not increase neurite outgrowth compared with the DRG of the control group, whereas DRG cells isolated from the FDR + CAN group showed an increase in neurite outgrowth similar to control DRG cells (Fig. 3B, bottom). Effect of the PI3K inhibitor on AT2R-mediated neurite outgrowth in cultured control DRG neurons. Next, we asked if insulin resistance induces the down-regulation of AT2R function. We focused on the PI3K-Akt
Because we found AT2R-mediated neurite outgrowth function was downregulated in the DRG of the FDR group, we examined whether PI3K inhibition could affect AT2R function in control rat DRG cells. Treatment with the PI3K inhibitor LY294002 (50 μmol/L) significantly inhibited neurite elongation after AT2R activation with CGP42112 similar to AT2R-mediated neurite outgrowth in cultured FDR DRG cells (Fig. 4; P = 0.0013).

**Insulin resistance downregulates Akt activity via a PI3K-dependent pathway in DRG neurons.** Because PI3K inhibition, which results in insulin resistance (13), was able to inhibit the function of AT2Rs, we next aimed to determine the mechanism. Akt is activated by insulin or various growth factors to function in a PI3K-sensitive pathway. Therefore, we examined the expression of phosphorylated Akt in control rats and FDR DRG neurons. We found
that expression of phosphorylated Akt in FDR cells was significantly reduced by ~20% when compared with control rats (Fig. 5A; P = 0.0079).

To confirm the involvement of PI3K, we examined the effect of the PI3K activator YS49 (100 nmol/L and 1 µmol/L) on AT2R-mediated neurite outgrowth in cultured DRG neurons isolated from the FDR group. The PI3K activator YS49 (100 nmol/L and 1 µmol/L) slightly increased neurite outgrowth after AT2R activation with CGP42112 (10 nmol/L) in cultured DRG cells isolated from the FDR group (Fig. 5B). Furthermore, the level of phosphorylated Akt expression in cultured FDR DRG cells subjected to AT2R activation using CGP42112 (10 nmol/L) in the presence of the PI3K activator YS49 (1 µmol/L) was significantly increased by ~35% in its absence (Fig. 5C; P = 0.0112).

**AT1R blockade increases phosphorylated Akt expression in DRG cells.** We next determined whether blockade of the AT1R prevented a decrease in phosphorylated Akt in the FDR group. Our results showed that DRG cells isolated from the FDR group had reduced expression of phosphorylated Akt compared with the control (see Fig. 5A). Administration of the AT1R antagonist candesartan cilexetil in the FDR group significantly increased phosphorylated Akt expression by ~44% (Fig. 6; P = 0.0453). Based on these results, we propose that blockade of the AT1R induces phosphorylation of Akt and improves AT2R function.

**DISCUSSION**

In the current study, we demonstrated that insulin resistance induced peripheral sensory nerve defects, delayed the response to noxious thermal stimuli, and reduced the density of CGRP-LI nerve fibers in mesenteric arteries, which were improved after administration of the AT1R antagonist candesartan cilexetil. We also showed that insulin resistance resulted in an increase of AT2R expression and dysfunction, which is related to depressed PI3K-dependent Akt activation.
In the current study, we used FDR, which have been established as an animal model of insulin resistance, and it mimics human metabolic syndrome in many aspects, including hyperinsulinemia and hypertriglyceridemia (14). In our protocol, 6-week-old rats were given 15% (w/v) fructose solution with or without candesartan cilexetil for 4 weeks. At 10 weeks, when compared with age-matched control rats, fasting blood glucose levels were not different but plasma insulin levels were significantly higher in the FDR group (see Table 1). Moreover, during the oral glucose tolerance test, increases in blood glucose and insulin levels were reduced to control rat levels after candesartan

FIG. 5. Insulin resistance downregulates Akt activity via a PI3K-dependent pathway in DRG neurons. A: Representative Western blots of Akt phosphorylation (p-Akt) and Akt isolated from DRG cells of the control (white bar) and FDR (black bar) groups. Data are the mean ± SEM of normalized densitometry measurements from Western blots of p-Akt compared with Akt (n = 5 to 6). B: Primary FDR DRG neuron cultures were incubated with the AT2R activator CGP42112 (10 nmol/L) only (white bar) or CGP42112 + the PI3K activator YS49 (100 nmol/L or 1 μmol/L; black bars) for 4 days. The bar graph represents the change in mean neurite length after treatment with each drug. Each bar indicates the mean ± SEM of three independent experiments. C: Representative Western blots of p-Akt and Akt from primary FDR DRG neuron cultures, which were incubated with CGP42112 (10 nmol/L) alone (white bar) or CGP42112 + YS49 (1 μmol/L; black bar) for 4 days. Data are the mean ± SEM of normalized densitometry measurements from Western blots of p-Akt compared with Akt (n = 3–5).
clarify the effect of candesartan treatment on triglycerides Zucker fatty rats (17). Further study will be needed to blocker) did not in diabetes.diabetesjournals.org DIABETES 7
cilexetil treatment. These
FIG. 6. AT1R blockade increases Akt phosphorylation (p-Akt) expres-
bar) groups are shown. Data are the mean ± SEM of normalized den-
sitometry measurements from Western blots of p-Akt compared with
Akt (n = 4).
cilexetil treatment. These findings suggest that AT1R block-
ade improved insulin sensitivity in fructose-induced insulin-
resistant rats.

Hyperglycemia is known to contribute significantly to
damage of nerve tissue in diabetes by the increased flux of
glucose through the sorbitol/aldose reductase pathway. However, as shown by our present study, thermal hypo-
algesia and a decrease of CGRP-LI sensory nerve in-
nervation were observed in the FDR group, which had
hyperinsulinemia but not hyperglycemia. Our laboratory
has reported previously a reduction in microvascular
function of rat mesenteric arteries and perivascular nerve
dysfunction in FDR (9). Moreover, recent evidence de-
monstrated that obese Zucker diabetic fatty rats, a model for
type 2 diabetes, developed vascular and neural impairment
independently of hyperglycemia (15). Consistent with this,
the current study suggests that not only hyperglycemia
but also insulin resistance may contribute to diabetes-
like neuropathy.

In the current study, we did not measure plasma trigly-
cerides level. There is one possibility that hyperlipidemia
could be associated with the insulin resistance–mediated
nerve dysfunction through circulating. Another related
study has demonstrated that irbesartan (AT1R blocker)
reduced triglycerides level in the insulin-resistant rat (16)
but another study has shown that olmesartan (AT1R
blocker) did not influence the plasma triglycerides level in
Zucker fatty rats (17). Further study will be needed to
clarify the effect of candesartan treatment on triglycerides
level and whether hyperlipidemia is involved in sensory
nerve defect by insulin resistance or not.

The AT2R is known to mediate the effects of nerve re-
generation. Our previous report suggested that AT2R ac-
tivation facilitated the density of CGRP-LI nerves in rat
esenteric arteries (10). In the current study, we showed
that insulin resistance induced the impairment of AT2R-
mediated neurite outgrowth function. Of interest, treat-
ment with the PI3K activator YS49 resulted in recovered
downregulation of AT2R neuronal function in insulin-
resistant FDR DRG neurons. Furthermore, the PI3K acti-
vator YS49 increased phosphorylation of Akt with AT2R
activation in FDR DRG neurons. These results suggest that
AT2R-mediated neurite outgrowth function may be re-
quired for PI3K and Akt activation. However, Cui et al. (18)
demonstrated that AT2R activation inhibited insulin-
duced PI3K activation and Akt phosphorylation inducing
apoptosis in PC12 cells. The discrepancy between their
results and ours may be caused by differences in exper-
imental conditions (cell line or primary cell culture) and/or
AT2R function (apoptosis or neurite outgrowth).

We have demonstrated for the first time that candesartan
cilexetil treatment improves the hypoalgesic response to a
thermal stimulus, innervation of CGRP-LI sensory nerves,
and AT2R downregulation in a rat model of fructose-
induced insulin resistance. In both clinical and experimen-
tal studies, AT1R antagonists have been shown to improve
insulin resistance (19–22). Ang II is a well-known inhibitor
of insulin signaling at various levels, including insulin re-
ceptor substrates and PI3K-Akt in vascular smooth muscle
cells or skeletal muscle (19,23,24). Another related study
demonstrated that candesartan treatment restored insulin-
mediated PI3K/Akt phosphorylation in Dahl salt-sensitive
rat aortas (25). Moreover, the PI3K-Akt signaling pathway
promotes neurite elongation in PC12 cells (26,27). Our data
showed that administration of candesartan cilexetil in-
creased the expression of phosphorylated Akt in FDR
DRG neurons to control levels. These results suggest that
PI3K-dependent Akt expression plays an important role in
AT1R-mediated blockade of neuronal improvement.

However, the exact mechanism of how AT1R blockade re-
stores AT2R expression and function in FDR remains
unclear. You et al. (28) showed that candesartan treatment
restored AT2R expression and vasodilator function in
spontaneously hypertensive rats. One possible mechanism
involves blockade of the AT1R, which leaves the AT2R
open to stimulation by Ang II, suggesting that preventing
the action of Ang II via the AT1R allows free AT2Rs to
respond to Ang II during neurite outgrowth. AT2Rs have
two neuronal differentiation pathways; one is the NO-
cGMP-PKG pathway and the other is the Rap-Raf-MEK-ERK
cascade (29). Akt is known to regulate the phosphoryla-
tion of Raf (30). Our data showed an increase of AT2R
expression, but function was attenuated in FDR, indicat-
ing that FDR had an impaired AT2R signaling cascade.
Therefore, we focused on PI3K-Akt pathway. Based on
our present data demonstrating increasing Akt phos-
phorylation in DRG neurons after candesartan treat-
ment with the PI3K activator YS49 resulted in recovered
mediated neurite outgrowth function. Of interest, treat-
mantion was attenuated in fructose-induced insulin-
resistant FDR (9). Moreover, recent evidence dem-
demonstrated that candesartan treatment restored insulin-
function of rat mesenteric arteries and perivascular nerve
dysfunction in FDR (10). In the current study, we showed
that insulin resistance induced the impairment of AT2R-
mediated neurite outgrowth function. Of interest, treat-
mantion was attenuated in fructose-induced insulin-
resistant FDR (9). Moreover, recent evidence dem-
demonstrated that candesartan treatment restored insulin-
rates in the FDR (10). In the current study, we showed
that insulin resistance induced the impairment of AT2R-
mediated neurite outgrowth function. Of interest, treat-
mantion was attenuated in fructose-induced insulin-
resistant FDR (9). Moreover, recent evidence dem-
demonstrated that candesartan treatment restored insulin-
function of rat mesenteric arteries and perivascular nerve
dysfunction in FDR (10). In the current study, we showed
that insulin resistance induced the impairment of AT2R-
mediated neurite outgrowth function. Of interest, treat-
mantion was attenuated in fructose-induced insulin-
resistant FDR (9). Moreover, recent evidence dem-
demonstrated that candesartan treatment restored insulin-
rates in the FDR (10). In the current study, we showed
that insulin resistance induced the impairment of AT2R-
mediated neurite outgrowth function. Of interest, treat-
mantion was attenuated in fructose-induced insulin-
resistant FDR (9). Moreover, recent evidence dem-
demonstrated that candesartan treatment restored insulin-
rates in the FDR (10). In the current study, we showed
that insulin resistance induced the impairment of AT2R-
mediated neurite outgrowth function. Of interest, treat-
mantion was attenuated in fructose-induced insulin-
resistant FDR (9). Moreover, recent evidence dem-

group, which was associated with the restoration of Akt activation and AT₃R function.

ACKNOWLEDGMENTS

This study was supported in part by a grant-in-aid for Scientific Research (KAKENHI) (No 21770192) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

No potential conflicts of interest relevant to this article were reported.

N.H.-H. designed and conducted experiments, analyzed data, and wrote the manuscript. N.H. analyzed the data, contributed to discussions, and reviewed the manuscript. Y.I. performed animal treatments. H.S. provided technical assistance. Y.Z. analyzed the data and contributed to discussions. S.T. and H.K. reviewed the manuscript. N.H.-H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The authors thank Shinonaga of Edanz Corporation and American Journal Experts for help in the preparation of this manuscript.

REFERENCES

1. Folli F, Kahn CR, Hansen H, Bouchie JL, Freener EP. Angiotensin II inhibits insulin signaling in aortic smooth muscle cells at multiple levels. A potential role for serine phosphorylation in insulin/angiotensin II crosstalk. J Clin Invest 1997;100:2158–2169
2. Shiuchi T, Iwai M, Li HS, et al. Angiotensin II type-1 receptor blocker valsartan enhances insulin sensitivity in skeletal muscles of diabetic mice. Hypertension 2004;43:1003–1010
3. Wei Y, Sowers JR, Nistala R, et al. Angiotensin II-induced NADPH oxidase activation impairs insulin signaling in skeletal muscle cells. J Biol Chem 2006;281:35137–35146
4. Igarashi M, Hirata A, Nozaki H, Kadomoto-Antsuki Y, Tominaga M. Role of angiotensin type 2 receptor in the development of the cardiovascular system of the rat. Hypertension 1996;28:91–97
5. Sales VL, Sukhova GK, Lopez-Illasaca MA, Libby P, Dzau VJ, Pratt RE. Angiotensin type 2 receptor is expressed in murine atherosclerotic lesions and modulates lesion evolution. Circulation 2005;112:3328–3336
6. Stroth U, Meffert S, Gallinat S, Unger T. Angiotensin II and NGF differentially influence microtubule proteins in PC12W cells: role of the AT2 receptor. Brain Res Mol Brain Res 1998;53:187–195
7. Takatori S, Zamami Y, Yabunaka N, et al. Pioglitazone opposes neurogenic vascular dysfunction associated with chronic hyperinsulinemia. Br J Pharmacol 2008;153:1388–1398
8. Zamami Y, Takatori S, Hoshara N, et al. Hyperinsulinemia induces hypertensive effects and angiotensin II and NGF differentially influence microtubule proteins in PC12W cells: role of the AT2 receptor. Brain Res Mol Brain Res 1998;53:187–195
9. Takatori S, Zamami Y, Yabunaka N, et al. Pioglitazone opposes neurogenic vascular dysfunction associated with chronic hyperinsulinemia. Br J Pharmacol 2008;153:1388–1398
10. Zamami Y, Takatori S, Hoshara N, et al. Hyperinsulinemia induces hypertensive effects and angiotensin II and NGF differentially influence microtubule proteins in PC12W cells: role of the AT2 receptor. Brain Res Mol Brain Res 1998;53:187–195
11. Read DE, Gorman AM. Involvement of Akt in neurite outgrowth. Cell Mol Life Sci 2009;66:2975–2984
12. Edly NB, Leimbach D. Synthetic analogues. II. Ditienylbutenyl- and dihydrobutilamines. J Pharmacol Exp Ther 1953;107:385–393
13. Zawalich WS, Zawalich KC. A link between insulin resistance and hyperinsulinemia: inhibitors of phosphatidylinositol 3-kinase augment glucose-induced insulin secretion from islets of lean, but not obese, rats. Endocrinology 2000;141:3287–3295
14. Thorburn AW, Storlien LH, Jenkins AB, Khouri S, Kraegen EW. Fructose-induced in vivo insulin resistance and elevated plasma triglyceride levels in rats. Am J Clin Nutr 1989;49:1155–1163
15. Oltman CL, Coppey LJ, Gellett JS, Davidson EP, Lund DD, Yorek MA. Progression of vascular and neural dysfunction in sciatic nerves of Zucker diabetic fatty and Zucker rats. Am J Physiol Endocrinol Metab 2005;289:E113–E122
16. Russell JC, Kelly SE, Vine DF, Proctor SD. Irbesartan-mediated reduction of renal and cardiac damage in insulin resistant JCR : LA-AT rats. Br J Pharmacol 2009;158:1588–1596
17. Ran J, Hirano T, Adachi M. Angiotensin II type I receptor blocker ameliorates overproduction and accumulation of triglyceride in the liver of Zucker fatty rats. Am J Physiol Endocrinol Metab 2004;287:E227–E232
18. Cui TX, Nakagami H, Nahmias C, et al. Angiotensin II subtype 2 receptor activation inhibits insulin-induced phosphoinositide 3-kinase and Akt and induces apoptosis in PC12W cells. Mol Endocrinol 2002;16:2113–2123
19. Igarashi K, Ura N, Miyazaki Y, Shimamoto K. Effect of an angiotensin II receptor antagonist, candesartan, on insulin resistance and pressor mechanisms in essential hypertension. J Hum Hypertens 1999;13(Suppl 1): S71–S77
20. Clasen R, Schupp M, Foryst-Ludwig A, et al. PPARgamma-activating angiotensin type-I receptor blockers induce adiponectin. Hypertension 2005;46:137–143
21. Pachner S, Heemann U, Frank H. Effect of Renin-Angiotensin system blockade on insulin resistance and inflammatory parameters in patients with impaired glucose tolerance. Diabetes Care 2010;33:914–919
22. Sukumoboon N, Poolsp N, Pratit S. Systematic review of the effect of telmisartan on insulin sensitivity in hypertensive patients with insulin resistance or diabetes. J Clin Pharm Ther. In press
23. Tanigaya Y, Hitomi H, Shah A, Alexander RW, Griending KK. Mechanisms of reactive oxygen species-dependent downregulation of insulin receptor substrate-1 by angiotensin II Arterioscler Thromb Vasc Biol 2005;25:1142–1147
24. Cribi A, Communi D, Muller N, Bottari SP. Angiotensin II inhibits insulin-stimulated GLUT4 translocation and Akt activation through tyrosine nitration-dependent mechanisms. PLoS ONE 2010;5:e10070
25. Zhou MS, Schulman IH, Raja L. Role of angiotensin II and oxidative stress in vascular insulin resistance linked to hypertension. Am J Physiol Heart Circ Physiol 2009;296:H833–H839
26. Kimura K, Hattori S, Kabuyama Y, et al. Neurite outgrowth of PC12 cells is induced in vivo insulin resistance and elevated plasma triglyceride levels in rats. Am J Physiol Endocrinol Metab 2005;289:E227–E232
27. Kim Y, Seger R, Suresh Babu CV, Hwang SY, Yoo YS. A positive role of the AT2 receptor antagonist, candesartan, on insulin resistance and pressor mechanisms in essential hypertension. J Hum Hypertens 1999;13(Suppl 1): S71–S77
28. Eddy NB, Leimbach D. Synthetic analgesics. II. Dithienylbutenyl- and dihydrobutilamines. J Pharmacol Exp Ther 1953;107:385–393
29. Gendron L, Payet MD, Gallo-Payet N. The angiotensin type 2 receptor of rat brain. Hypertension 2004;43:1003–1010
30. Zimmermann S, Moelling K. Phosphorylation and regulation of Raf by Akt in PC12W cells. Mol Endocrinol 2002;16:2113–2123
31. Razumiuc I, Kuriyan J, Mount DB, et al. Dean and colleagues. A mechanism of angiotensin II receptor antagonism. Endocrinology 1999;140:472–479
32. Willemsen JF, de Vries AM, Brouwer DC, et al. A role for serine phosphorylation in insulin/angiotensin II crosstalk. J Clin Invest 1997;100:2158–2169
33. Shih SC, Lehrer I, Backlund KE, et al. A role for serine phosphorylation in insulin/angiotensin II crosstalk. J Clin Invest 1997;100:2158–2169

Candesartan improves neurite outgrowth