Published in final edited form as:

Eur J Pharmacol. 2010 July 25; 638(1-3): 108–114. doi:10.1016/j.ejphar.2010.04.030.

Angiotensin-(1–7) prevents diabetes-induced attenuation in PPAR- γ and catalase activities

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Abstract

The mechanisms by which angiotensin-(1–7) [Ang-(1–7)] exerts its beneficial effects on end-organ damage associated with diabetes and hypertension are not well understood. The purpose of this study was A) to compare the effects of apocynin with Ang-(1–7) on renal vascular dysfunction and NADPH oxidase activity in a combined model of diabetes and hypertension and B) to further determine whether chronic treatment with Ang-(1–7) can modulate renal catalase, and peroxisome proliferator activated receptor- γ (PPAR- γ) levels in streptozotocin induced-diabetes in both normotensive Wistar Kyoto rats (WKY) and in spontaneously hypertensive rats (SHR). Apocynin or Ang-(1–7) treatment for one month starting at the onset of diabetes similarly attenuated elevation of renal NADPH oxidase activity in the diabetic SHR kidney and reduced the degree of proteinuria and hyperglycemia, but had little or modest effect on reducing mean arterial pressure. Both drugs also attenuated the diabetes-induced increase in renal vascular responsiveness to endothelin-1. Induction of diabetes in WKY and SHR animals resulted in significantly reduced renal catalase activity and in PPAR- γ mRNA and protein levels. Treatment with Ang-(1–7) significantly prevented diabetes-induced reduction in catalase activity and the reduction in PPAR- γ mRNA and protein levels in both animal models. Taken together, these data suggest that activation of Ang-(1–7)-mediated signaling could be an effective way to prevent the elevation of NADPH oxidase activity and inhibition of PPAR- γ and catalase activities in diabetes and/or hypertension.

Keywords

Angiotensin; hypertension; diabetes; kidney

1. Introduction

Reactive oxygen species are important players in routine physiological and cellular processes including salt and fluid homeostasis, cell growth and apoptosis (Nistala et al.,

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2008; Wolin 2009). However, overproduction of free radicals or reactive oxygen species are increasingly implicated to have causal roles in the development of end-organ pathologies associated with diabetes and/or hypertension (Frey et al., 2009; Houstis et al., 2006; Nistala et al., 2008; Wolin 2009). Markers of reactive oxygen species, especially increased H_2O_2 , are thought to be linked with increased incidence of diabetes and its associated end-organ pathologies (Góth 2008; Góth L and Eaton, 2000). An important antioxidant enzyme is catalase, which is found in the liver, kidney and blood. Overexpression of catalase prevented the stimulation of reactive oxygen species and angiotensinogen mRNA in kidney tubules in response to elevated glucose or angiotensin II (Ang II) (Breznicianu et al., 2008). Additionally, overexpression of catalase attenuated reactive oxygen species generation, angiotensinogen and proapoptotic gene expression and apoptosis in the kidneys of diabetic mice suggesting an important role of reactive oxygen species in the pathophysiology of diabetic nephropathy (Breznicianu et al., 2007 and 2008).

Peroxisome proliferator activator receptors (PPARs) are a novel class of nuclear receptors that can modulate redox homeostasis and thus have been linked to development and progression of several diseases including cardiovascular and renovascular disorders (Ruan et al., 2008; Smeets et al., 2007). Activation of PPAR- γ has been reported as the target of several therapeutic agents including thiazolidinediones (or glitazones) that are used in the treatment of diabetes (Etgen and Mantlo 2003; Fogo 2008; Gervois et al., 2004). Activators of PPAR- γ such as glitazones that were originally established as insulin sensitizers, are now known to reduce hypertension, Ang II-induced oxidative stress, production of inflammatory markers such as NF- κ B and improve dyslipidemia in patients with type II diabetes (Fogo et al., 2008; Duan et al., 2008; Efrati et al., 2008; Yousefipour et al., 2007; Touyz and Schiffrin, 2006). Moreover, angiotensin converting enzyme (ACE) inhibitors and angiotensin type-1 (AT_1) receptor blockers especially telmisartan (and to a lesser degree candesartan and irbesartan) have been reported to partially increase PPAR- activity (Erbe et al., 2006; Fogo et al., 2008; Kurtz 2008; Kurtz and Pravenee, 2004; Unger and Stoppelhaar, 2007; Yamagishi et al., 2008).

Angiotensin-(1-7) [Ang-(1-7)] is a vasodilator peptide that exhibits antihypertensive, antithrombotic and antiproliferative properties (Benter et al., 1993 and 2006; Chappell 2007). In fact, the effects of ACE inhibitors and angiotensin AT_1 receptor blockers may be mediated, at least in part, through Ang-(1-7) (Chappell 2007). The effects of Ang-(1-7) are mediated through the G protein-coupled receptor *mas* and involve activation of vasodilatory prostaglandins and nitric oxide (Benter et al., 1993 and 2006; Chappell 2007). Decreasing Ang-(1-7) synthesis by inhibiting ACE2 results in kidney damage (Chappell 2007; Soler et al., 2007). In addition, exogenous Ang-(1-7) reduces end-organ damage in models of diabetes and/or hypertension (Benter et al., 2006 and 2007). Indeed, we recently reported that Ang-(1-7) prevents renal dysfunction in diabetic hypertensive rats through inhibition of renal NADPH oxidase (Benter et al., 2008). The current study compared the effects of apocynin, a known antioxidant, with Ang-(1-7) in a combined model of diabetes and hypertension and further examined the effect of Ang-(1-7) on renal PPAR- γ expression and catalase activity.

2. Materials and Methods

2.1 Materials and Chemical Reagents

Streptozotocin, endothelin-1, Ang-(1-7) and apocynin were procured from Sigma-Aldrich (St. Louis, MO., U.S.A.) whereas all other chemicals were obtained from Calbiochem (La Jolla, CA., U.S.A.). Reagents and supplies used for electrophoresis were purchased from Bio-Rad (Hercules, CA., U.S.A.).

2.2 Animals and Treatment groups

Two separate animal studies were performed. Male Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR) were used in both studies.

A) To study the effect of apocynin and Ang-(1–7) treatment on NADPH oxidase activity and renal vascular reactivity in a model of combined diabetes and hypertension, rats were divided into the following groups (N=12/group). **Group 1:** Normotensive non-diabetic controls (WKY); **Group 2:** Hypertensive non-diabetic controls (SHR); **Group 3:** Hypertensive diabetic controls (SHR-STZ); **Group 4:** Hypertensive non-diabetic rats treated with apocynin (5 mg/kg/day *i.p.*) (SHR-apocynin); **Group 5:** Hypertensive diabetic rats treated with apocynin (5 mg/kg/day *i.p.*) (SHR-STZ-apocynin); **Group 6:** Hypertensive diabetic rats treated with Ang-(1–7) (576 µg/kg/day *i.p.*) (SHR-STZ-Ang-(1–7)). Diabetes was induced by a single *i.p.* injection of streptozotocin. Treatments with apocynin or Ang-(1–7) were initiated on the same day as streptozotocin injection and continued for 4 weeks at which point the animals were sacrificed for the stated studies. The doses of the Ang-(1–7) and apocynin were chosen on the basis of previous studies in models of hypertension and/or diabetes (Asaba et al., 2005, Benter et al., 2006, 2007 and 2008, Hayashi et al., 2005).

B) To study the effect of chronic treatment with Ang-(1–7) on renal catalase activity and PPAR-γ levels, animals were divided into eight groups (N=12/group): **Group 1:** Normotensive non-diabetic controls (WKY); **Group 2:** Hypertensive non-diabetic controls (SHR); **Group 3:** Normotensive diabetic controls (WKY-STZ); **Group 4:** Hypertensive diabetic controls (SHR-STZ); **Group 5:** Normotensive non-diabetic rats treated with Ang-(1–7) (576 µg/kg/day *i.p.*) [WKY-Ang-(1–7)]; **Group 6:** Hypertensive non-diabetic rats treated with Ang-(1–7) (576 µg/kg/day *i.p.*) [SHR-Ang-(1–7)]; **Group 7:** Normotensive diabetic rats treated with Ang-(1–7) (576 µg/kg/day *i.p.*) [WKY-STZ-Ang-(1–7)] and **Group 8:** Hypertensive diabetic rats treated with Ang-(1–7) (576 µg/kg/day *i.p.*) [SHR-STZ-Ang-(1–7)]. Animals were treated for four weeks with daily *i.p.* injections of vehicle or Ang-(1–7) and sacrificed at the end of the four week treatment period. Treatment with vehicle or Ang-(1–7) was initiated on the same day as streptozotocin injection as per the first study.

This investigation conforms to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1985) and is approved by Kuwait University Research Administration as the use of animals was in accordance with Institute for Laboratory Animal Research Guide for Care and Use of Laboratory Animals.

2.3 Induction of diabetes and blood glucose measurements

A single *i.p.* injection of streptozotocin (55 mg/kg body weight) dissolved in citrate buffer (pH 4.5) was used to induce diabetes. Establishment of diabetes was verified by blood glucose concentrations above 200mg/dl 48h after streptozotocin injection. Blood glucose levels were measured using an automated blood glucose analyzer (Glucometer Elite XL). The levels of blood glucose and body weight were re-assessed after 4 weeks just before sacrificing the animals.

2.4 Urine analysis

In order to determine the urinary volume, protein and lysozyme concentration at the end of the 4-weeks treatment period, animals were placed in metabolic cages. Food and water were provided *ad libitum*. Collection of urine samples was carried out for 24 h. Total protein and lysozyme concentration in the urine were determined as reported previously (Benter et al., 2006).

2.5 Blood Pressure Measurement

The animals were anaesthetized with sodium pentobarbital (60 mg/kg) and the mean arterial pressure was measured. A catheter was inserted into the left femoral artery and was connected to a pressure transducer for blood pressure measurement as described by us previously (Benter et al., 2006).

2.6 NADPH oxidase activity studies

Kidneys were removed from the euthanized animals at the end of the four-week treatment period. One part of the kidney cortex was used to prepare tissue homogenates in 0.25 M sucrose buffer, pH 7.2. Kidney homogenates were used for assay of NADPH oxidase activity. The other half of renal cortex was immediately frozen in liquid nitrogen and was used to isolate RNA. NADPH oxidase activity was measured in tissue homogenates at room temperature in assay mixture that contained 50 mM phosphate buffer, pH 7.1, 0.01 mM EDTA and 250 μ M lucigenin. The tissue homogenate was first added to the reaction mixture to reach a point of equilibrium (3–5 min) that there was no chemiluminescence, and then the reaction was started by addition of 100 μ M of NADPH. Chemiluminescence in response to NADPH addition was recorded over a period of 3 min in the presence or absence of 25 μ M diphenyliodonium, an inhibitor of NADPH oxidase. Diphenyliodonium inhibitable activity was taken as the NADPH oxidase activity and calculated as relative light units (RLU) emitted per sec per mg of protein.

2.7 RT-PCR detection of PPAR- γ and GAPDH mRNA

In each experiment, total RNA was extracted from kidney cortex with RNA extraction kit based on use of guanidinium thiocyanate lithium chloride and cesium trifluoroacetate. Isolated RNA was of high quality and was used immediately for synthesis of first strand cDNA according to protocols from Clontech's SMART PCR cDNA synthesis kit. Amplification of cDNA obtained from reverse transcription of RNAs was carried out using Advantage cDNA PCR kit (BD Biosciences Clontech) and the following primers for PPAR- γ ; sense, 5'-CCATTCTGGCCCAAC-3' and antisense, 5'-CTGAAACCGACAGTACTG-3'. Primers for GAPDH were provided by Clontech. First strand of cDNA obtained from reverse transcription was denatured for 1 min at 95°C and subjected to PCR with following parameters; 95°C for 30sec, 58°C or 62°C for 30sec, 68°C for 45 sec, 25–30 cycles after denaturing at 95°C for 1min. PCR products were then analyzed using agarose gel electrophoresis.

2.8 Western blotting for PPAR- γ

Kidney cortex homogenates were prepared in Tris-HCl Buffer (50mM) pH 7.4 and cellular proteins were extracted using lysis buffer (50 mM Tris-HCl, 15 mM EGTA, 100 mM NaCl, 0.1% (w/v) Triton X-100 and protease inhibitor cocktail). Protein lysates were subjected to Polyacrylamide Gel Electrophoresis followed by transfer of proteins to nitrocellulose membrane. Immunoblotting was performed using rabbit polyclonal antibodies against PPAR gamma peptide of human origin (Santa Cruz Biotech. Inc.). A horseradish peroxidase-labeled secondary antibody and ECL reagents were used to detect the protein bands.

2.9 Catalase activity assay

Homogenates of kidney cortex were prepared in 0.25 M sucrose buffer, pH 7.2, and used for assays of catalase as described earlier (Baudhuin et al., 1964; Small et al., 1985). Catalase activity was assayed by measuring the degradation of hydrogen peroxide in the presence or absence of aminotriazole, a specific irreversible inhibitor of catalase. Protein was measured in tissue homogenates and urine samples using Bio-Rad kit (catalogue # 500-0006).

2.10 Vascular Reactivity Experiments

The animals were sacrificed at the end of the four-week study by decapitation under ether anesthesia. The renal artery was isolated carefully and ring segments of 5 mm were mounted in organ-baths containing 25 ml Krebs-Henseleit solution at pH 7.4. The composition of Krebs-Henseleit solution is as follows (mM): NaCl (118.3), KCl (4.7), CaCl₂ (2.5), MgSO₄ (1.2), NaHCO₃ (25), KH₂PO₄ (1.2) and glucose (11.2). The vessels were mounted in a bath solution which was set at 37°C and aerated with 95% oxygen and 5% carbon dioxide. Responses of the renal arteries to the vasoactive agonist were measured using UFI dynamometers on a Lectromed 2-channel recorder. A pretension of 0.5 g was applied and the preparations were left until a stable baseline tone was obtained (for about 45 min).

A cumulative concentration response curve was established for endothelin-1 following the period of equilibration. The vasoconstrictor effect of endothelin-1 was investigated on isolated renal artery ring segments from all the animal groups included in this study. The different doses of endothelin-1 (10^{-11} , 10^{-10} , 10^{-9} and 10^{-8} M) were added successively to the organ-bath to establish the vasoconstrictor responses. Each concentration of the agonist was tested until a stable response was obtained. Thereafter, the next concentration of the agonist was tested. It required about 30–40 minutes for each concentration of the drug in order to get the plateau effect.

2.11 Statistical analysis

The data are presented as mean $\pm$ S.E.M. Statistical significance was assessed by one- or two-way ANOVA followed by Bonferroni posttests (GRAPHPAD Prism 4; GraphPad Software, Inc., San Diego, CA., U.S.A.). Values of $P < 0.05$ were considered significant.

3. Results

We had previously shown that in a combined model of diabetes and hypertension, treatment with Ang-(1–7) prevented diabetes-induced elevation in NADPH oxidase activity (Benter et al., 2008). In the results below, we have now **A**) compared the effects of apocynin, a known antioxidant and inhibitor of NADPH oxidase, with Ang-(1–7) treatment in a combined model of diabetes and hypertension and **B**) have examined the effect of Ang-(1–7) treatment on catalase activity and PPAR- γ levels in kidneys of rats with diabetes and/or hypertension.

3.1 Effect of apocynin and Ang-(1–7) treatment on diabetic SHR animals

3.1.1 Hyperglycemia and body weight—A significant increase in blood glucose concentration was observed after inducing diabetes by streptozotocin in SHR (Table 1). The blood glucose concentration in diabetic SHR was modestly but statistically significantly reduced (~ 15% and 25%) upon treatment with apocynin or with Ang-(1–7) respectively ($P < 0.05$). However the values were still greatly elevated (i.e. animals remained hyperglycemic) compared to non-diabetic controls. The body weights of SHR were significantly reduced (~23%) after induction of diabetes. Treatment of diabetic SHR with apocynin significantly increased body weight of diabetic animals by about 11% compared to 27% for Ang (1–7) treatment ($P < 0.05$; Table 1). Treatment with Ang-(1–7), but not apocynin, completely prevented hyperglycemia-induced weight loss of diabetic SHR as body weights were not significantly different to non-diabetic controls ($P > 0.05$; Table 1).

3.1.2 Mean arterial pressure—Table 1 shows a modest but statistically significant (~ 16%) reduction in mean arterial pressure for apocynin-treated SHR compared to SHR controls ($P < 0.05$). Induction of diabetes with streptozotocin resulted in a significant (~ 20%) attenuation of mean arterial pressure in SHR ($P < 0.05$). Mean arterial pressure was also reduced (~ 14%) in the diabetic SHR treated with apocynin compared to diabetic-SHR. In

contrast, treatment with Ang (1–7) had no significant effect on mean arterial pressure (Table 1).

3.1.3 Urinary protein, lysozyme and urine volume data—Urinary protein but not urine volume significantly increased in SHR compared to WKY animals and apocynin treatment did not prevent these changes (Table 1). Induction of diabetes in SHR increased both urine volume and urinary protein by ~800% and 180%, respectively. Treatment of diabetic SHR with apocynin or Ang (1–7) had no effect on urine volume but significantly attenuated urinary protein by approximately 50% in either treatment (Table 1). Furthermore, there was a significant increase in urinary lysozyme in diabetic SHR compared to WKY or SHR. Treatment with apocynin or Ang-(1–7) did not produce any reduction in the urinary lysozymes of diabetic SHR (Table 1).

3.1.4 Effect of apocynin on NADPH oxidase activity—Renal NADPH oxidase activity was elevated in SHR compared to WKY by about 45% (see Fig. 1). Induction of diabetes resulted in a further significant increase in NADPH oxidase activity in the kidneys of SHR ($P<0.01$) (Fig. 1). Apocynin or Ang-(1–7) treatment of diabetic-SHR reduced renal NADPH oxidase activity levels similar to those of WKY controls (Fig. 1). Furthermore, treatment of SHR with apocynin also reduced NADPH oxidase activity to that of normotensive WKY controls (Fig. 1).

3.1.5 Vascular reactivity experiments—Endothelin-1 produced a concentration-dependent vasoconstriction of the isolated renal artery segments. The vasoconstrictor response to endothelin-1 was significantly potentiated in SHR compared to WKY and upon induction of diabetes in SHR (Fig. 2). Both apocynin and Ang-(1–7) treatments of diabetic SHR had similar corrective effects on the exaggerated vasoconstrictive effects of endothelin-1 (Fig. 2). Treatment of non-diabetic SHR with either apocynin or Ang-(1–7) had no effect on the vascular reactivity to endothelin-1 (Fig. 2).

3.2 Effect of Ang-(1–7) on renal catalase activity and PPAR- γ expression

3.2.1 Effect of Ang-(1–7) on renal catalase activity in diabetic and/or hypertensive rats—Induction of diabetes led to a reduction in renal catalase activity that could be significantly prevented by Ang-(1–7) treatment in both WKY and SHR animal models (Fig. 3). Ang-(1–7) treatment led to complete recovery in WKY animals whereas in SHR about a 60% correction was observed in diabetes-induced reduction in renal catalase activity (Fig. 3). Interestingly, treatment with Ang-(1–7) led to increased catalase activity in non-diabetic WKY but not in non-diabetic SHR compared to their corresponding untreated controls (Fig. 3).

3.2.2 Effect of Ang-(1–7) on renal PPAR- γ expression and protein levels in diabetic- and/or hypertensive rats—Induction of diabetes lowered PPAR- γ mRNA levels in both WKY and SHR (Fig. 4). Treatment with Ang-(1–7) not only prevented the diabetes-induced reduction in PPAR- γ mRNA levels but significantly elevated its levels in all groups except WKY relative to the respective untreated controls. Interestingly, PPAR- γ mRNA levels were lower in non-diabetic SHR compared to non-diabetic WKY animals (Fig. 4a). A similar influence on renal PPAR- γ protein expression by exogenous Ang-(1–7) was observed (see immunoblot in Fig. 4b).

4. Discussion

The major finding of the present study is that exogenous treatment with Ang-(1–7) prevents diabetes-induced reductions in renal catalase activity and PPAR- γ protein expression within

the kidney. These novel findings reveal the mechanisms by which Ang-(1–7) may function to counteract the pro-oxidant effects that contribute to renal dysfunction in diabetes. We previously demonstrated that Ang-(1–7) treatment prevented the diabetes-induced elevation in NADPH oxidase activity in the same model of combined diabetes and hypertension (Benter et al., 2008). In the present study we demonstrate that Ang-(1–7) and the antioxidant, apocynin, a known inhibitor of reactive oxygen species generation, have comparable effects to attenuate the diabetes-induced elevation in NADPH oxidase activity in the kidney of diabetic hypertensive rats (STZ-SHR). In addition, apocynin-induced reduction in NADPH oxidase activity in STZ-SHR was accompanied by improvements in proteinuria, mean arterial pressure and body weight and in the vascular reactivity of renal artery.

We had previously shown in a model of diabetes that treatment with Ang-(1–7) or its synthetic non-peptide analog, AVE0991, prevented diabetes-induced proteinuria and vascular dysfunction in the renal artery, carotid artery and the mesenteric bed (Benter et al., 2007). We also showed that in a combined model of diabetes and hypertension that Ang-(1–7) prevented development of proteinuria and renal artery dysfunction associated with decreased renal NADPH oxidase activity and inhibition of NADPH oxidase-4 gene expression (Benter et al., 2008). The aim of the present study was to investigate further the antioxidant mechanisms that are associated with the Ang-(1–7) induced beneficial effects on the kidney in models of diabetes, hypertension and combined diabetes and hypertension.

In this study we firstly showed that Ang-(1–7) or apocynin treatment had similar effects on NADPH oxidase activity, proteinuria and renal artery reactivity leading us to further speculate that Ang-(1–7)-mediated renal protection, at least partially, involves a similar inhibition of NADPH oxidase and attenuation in reactive oxygen species generation. We also now report for the first time that treatment with Ang-(1–7) significantly prevented the diabetes-induced reduction in both catalase activity and PPAR- γ protein levels in the normotensive (WKY) and hypertensive (SHR) animal models of diabetes without a complete correction in hyperglycemia. Our results suggest that despite the persistence of hyperglycemia that normally leads to increased oxidative stress, Ang-(1–7) activates antioxidant pathways and prevents abnormal changes in key signaling molecules within the diabetes-induced oxidative stress pathway in the kidney of normotensive and hypertensive animal models.

Streptozotocin-induced diabetes in SHR caused a decrease in mean arterial pressure and body weight, an increase in blood glucose, proteinuria and renal NADPH oxidase activity. The reduction in mean arterial pressure following induction of diabetes in SHR is similar to what we and others have reported previously (Benter et al., 2008; Bidani et al., 2006) but mean arterial pressure is normally elevated following induction of diabetes in WKY animals and in patients with diabetes (Benter et al., 2008; Haidara et al., 2009; Kumagai et al., 2002). The explanation as to why mean arterial pressure is raised following induction of diabetes in WKY but not in diabetic SHR is unclear. It may reflect the presence of a compensatory response that opposes elevation of mean arterial pressure in the underlying state of hypertension following induction of diabetes but this hypothesis clearly needs further study.

The vascular reactivity to the vasoconstrictor endothelin-1 was also enhanced in the renal artery isolated from diabetic SHR. Renal NADPH oxidase activity was higher in diabetic WKY, SHR and diabetic SHR compared to untreated WKY. In diabetic SHR, renal NADPH oxidase activity was higher compared to SHR (Fig. 1). Chronic treatment with apocynin caused a significant reduction in mean arterial pressure in SHR and diabetic SHR (Table 1). Weight loss was significantly prevented following apocynin treatment in both diabetic WKY and diabetic SHR (Table 1). Further, treatment with apocynin significantly attenuated the

proteinuria in diabetic SHR (Table 1) and decreased the abnormal vascular reactivity in the renal artery of the diabetic WKY and diabetic SHR, in response to the constrictor agonist, endothelin-1 (Fig. 2). Interestingly, the beneficial effect of apocynin in decreasing the vascular contractions to endothelin-1 was significantly higher in the renal arteries obtained from animals with combined diabetes and hypertension, compared to the other animal groups in this study (Fig. 2). To our knowledge, this is the first study to show that the beneficial effect of apocynin in decreasing renal vascular dysfunction is significantly higher when diabetes and hypertension occur simultaneously.

Recent studies have shown that Ang-(1–7) acts as an intrinsic counterbalance to the detrimental actions of factors such as Ang II (Chappell 2007). We have reported that Ang-(1–7) prevents vascular, renal and cardiac dysfunction in rats with hypertension and/or diabetes (Benter et al., 2006 and 2007). Here we show that the elevated reactive oxygen species levels in the kidneys of normotensive and hypertensive animals with diabetes are counteracted by the antioxidant properties of Ang-(1–7) that mimic those of apocynin. While there may be several direct and indirect mechanisms for the effects of either the apocynin or the Ang-(1–7), the ability of both Ang-(1–7) and apocynin to prevent the diabetes-induced elevation in NADPH oxidase activity was associated with subsequent improvement in proteinuria and renal artery responsiveness to endothelin-1. Interestingly, apocynin and Ang-(1–7) treatments of diabetic SHR significantly prevented diabetes-induced weight loss but the effects with Ang-(1–7) were more pronounced implying that Ang-(1–7) might have additional beneficial effects or involve other mechanisms of action compared to apocynin. However, because of the improvements in weight loss, blood pressure, proteinuria and slight improvements in hyperglycemia, it is not possible to determine which factor(s) are direct or indirect contributors to the anti-oxidant effects observed.

PPAR- γ activators such as rosiglitazone, besides their effects on glycemic control have several other benefits such as blood pressure lowering, triglyceride reduction and high-density lipoprotein-cholesterol elevation (Campbell 2005; Duan et al., 2008). Pioglitazone, a PPAR- γ agonist, was shown to improve nitric oxide bioavailability in Ang II-infused rabbits (Imanishi et al., 2008). Ang II-induced activation of Bcr kinase in vascular smooth muscle cells results in increased NF κ B activation through inhibition of PPAR- γ activity (Alexis et al, 2009). Our findings in this study that Ang-(1–7) increases renal PPAR- γ mRNA and protein suggests that the previously reported actions of Ang-(1–7) on blood pressure, thrombosis and proliferation properties might be in part due to PPAR- γ activation. Numerous clinical trials have shown that inhibitors of Ang II (ACE and renin inhibitors and angiotensin AT₁ receptor blockers) reduce end-organ damage in patients with hypertension and/or diabetes. The mechanisms involved are not entirely clear but recent studies suggest that ACE inhibitors and angiotensin AT₁ receptor blockers reduce the hypertension and/or diabetes-related complications in part due to elevation of Ang-(1–7) levels and activation of PPAR- γ (Chappell 2007; Ikejima et al., 2008; Kurtz 2008; Kurtz and Pravenec, 2004). Telmisartan, an angiotensin AT₁ receptor blocker, inhibits advanced glycation end products-elicited endothelial cell injury by suppressing AGE receptor expression via PPAR- γ activation (Yamagishi et al., 2008). Further studies will be needed to determine the extent to which Ang-(1–7) contributes to the PPAR- γ agonistic effect of ACE inhibitors or angiotensin AT₁ receptor blockers.

Increasing attention has been devoted to developing catalase or peroxidase mimetics as a way to treat overt inflammation associated with the pathophysiology of many human disorders (Da Dos et al., 2005; Day 2009; Góth 2008; Góth L and Eaton, 2000). Catalytic antioxidants with H₂O₂-scavenging activities are effective in animal models of hypertension and diabetes (Brezniceanu et al., 2007). H₂O₂ at high concentrations is a toxic agent, while

at low concentrations it appears to modulate some physiological processes such as signaling in cell proliferation, apoptosis, carbohydrate metabolism, and platelet activation (Locatelli 2003). Catalase is responsible for sub-cellular breakdown of H_2O_2 and is markedly inhibited in kidneys of diabetic rats and this effect can be reversed by treatment with antioxidants (Locatelli 2003). We have reported that Ang-(1–7) exerts antioxidant effects through its inhibitory actions on superoxide anion generating NADPH oxidase enzyme system (Benter et al., 2008). Our findings in the present study that Ang-(1–7) attenuates the diabetes-induced inhibition of catalase enzyme activity further support the antioxidant capability of Ang-(1–7). Since Ang-(1–7) is known to down regulate NADPH oxidase activity in kidneys of diabetic rats, the observed increase in catalase activity might partially be due to the prevention of catalase inhibition by superoxide anion. Several PPAR-agonists such as ciprofibrate and clofibrate are well known to induce proliferation of peroxisomes including catalase.

In conclusion, stimulation of anti-oxidant pathways and modulation of PPAR- γ and catalase activities by Ang-(1–7) can be regarded as potential mechanisms for the beneficial actions of the peptide in hypertension and diabetes. Hence, potential strategies aimed at activating the Ang-(1–7)-PPAR- γ -catalase pathway represent promising novel approaches for the treatment of renal complications of diabetes and/or hypertension.

Perspectives

Increasing evidence supports a role of ACE2 as a protective component of the renal response to diabetes, since levels of the enzyme increase in streptozotocin-induced diabetes and deletion of the ACE2 gene or ACE2 inhibition worsens the renal effects in this model of diabetic renal injury (Koitka et al., 2008). These studies illustrate that the overall renal damage could be linked to either increases in Ang II or decreases in Ang-(1–7), emphasizing the pivotal role of the balance of these two peptide in dictating the impact of diabetes on the kidney. Our previous studies demonstrated a protective role for Ang-(1–7) exogenously administered in both hypertension and diabetes independently, and in the current study the combination of both pathologies were mitigated by administration of the peptide. Since it is well known that diabetes is accompanied by pro-oxidative stress induced renal injury, the ability of Ang-(1–7) treatment to shift renal pathways towards a better anti-oxidant profile presents a new mechanism to explain the beneficial effects of the peptide on proteinuria.

Acknowledgments

This work was supported by Kuwait University grant RM 02/03 and NIH grants HL-51952 and HL-56973. We would like to thank the late Dr. Constantin Cojocel for providing the data on urinary protein and lysozyme levels.

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Fig. 1.

Renal NADPH oxidase activity in WKY, SHR, SHR-STZ, SHR-apocynin, SHR-STZ-apocynin or SHR-STZ-Ang-(1-7). Enzyme activity is shown as percent of control. Values are Mean $\pm$ S.E.M., n=12 per group.

* significantly different compared to WKY, $P < 0.05$.

significantly different compared to SHR, $P < 0.05$.

† significantly different compared to SHR-STZ, $P < 0.05$.

Fig. 2. Endothelin-1-induced vasoconstriction (10^{-10} , 10^{-9} and 10^{-8} M) in the renal artery ring segments of WKY, SHR, SHR-STZ, SHR-apocynin, SHR-STZ-apocynin and SHR-STZ-Ang-(1-7). Values are Mean $\pm$ S.E.M., n=6 per group.

* significantly different compared to WKY, $P < 0.05$.

significantly different compared to SHR, $P < 0.05$.

† significantly different compared to SHR-STZ, $P < 0.05$.

§ significantly different compared to SHR-STZ-apocynin, $P < 0.05$.

Fig. 3.

Enzymatic activity of catalase (presented as percent of control) in kidneys of WKY, SHR, WKY-STZ, SHR-STZ, WKY-Ang-(1-7), SHR-Ang-(1-7), WKY-STZ-Ang-(1-7) and SHR-STZ-Ang-(1-7). Values are Mean $\pm$ S.E.M., n=4 per group.

* significantly different compared to WKY, $P < 0.05$.

significantly different compared to SHR, $P < 0.05$.

§ significantly different compared to WKY-STZ, $P < 0.05$.

† significantly different compared to SHR-STZ, $P < 0.05$.

Figure 4a

Figure 4b

Fig. 4.

RT-PCR analysis of mRNA levels of PPAR- γ (**a**) and protein levels by Western Blotting (**b**) in kidneys of WKY, SHR, WKY-STZ, SHR-STZ, WKY-Ang-(1–7), SHR-Ang-(1–7), WKY-STZ-Ang-(1–7) and SHR-STZ-Ang-(1–7). Bars show the ratio of mRNA levels of PPAR- γ and GAPDH for PCR and PPAR- γ and Actin in Western Blots. Values are Mean $\pm$ S.E.M., n=4 per group.

* significantly different compared to WKY, P<0.05.

significantly different compared to SHR, P<0.05.

§ significantly different compared to WKY-STZ, P<0.05.

† significantly different compared to SHR-STZ, P<0.05.

Table 1

Groups studied	Blood glucose(mg/dl)	Body weight(g)	Mean Arterial Pressure (mmHg)	Urine volume (ml/24h)	Urine Protein (mg/24h)	Urine Lysozyme (µg/24h)
1. WKY	90 ± 5	320 ± 9	121 ± 5	11 ± 2	75 ± 7	39 ± 4
2. SHR	97 ± 4	330 ± 7	207 ± 11 ^a	13 ± 4	115 ± 19 ^a	67 ± 8 ^a
3. SHR-STZ	570 ± 8 ^{a,b}	255 ± 10 ^{a,b}	166 ± 3 ^{a,b}	118 ± 5 ^{a,b}	322 ± 23 ^{a,b}	442 ± 21 ^{a,b}
4. SHR-apocynin	93 ± 3	365 ± 9 ^{a,b}	175 ± 3 ^{a,b}	18 ± 4	129 ± 19 ^a	68 ± 12 ^a
5. SHR-STZ-apocynin	486 ± 7 ^{a,b,c}	282 ± 4 ^{a,b,c}	142 ± 6 ^{a,b,c}	116 ± 5 ^{a,b}	152 ± 13 ^{a,b,c}	431 ± 19 ^{a,b}
6. SHR-STZ-Ang-(1-7)	430 ± 9 ^{a,b,c,d}	324 ± 8 ^{c,d}	160 ± 3 ^{a,b,d}	125 ± 4 ^{a,b}	176 ± 10 ^{a,b,c}	437 ± 25 ^{a,b}

Data presented as mean ± S.E.M., (N=6).

^aValue significantly different compared to WKY, P<0.05;

^bValue significantly different compared to SHR, P<0.05.

^cValue significantly different compared to SHR-STZ, P<0.05.

^dValue significantly different compared to SHR-STZ-apocynin, P<0.05.