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Endogenous angiotensin-(1–7) reduces cardiac ischemia-induced dysfunction in diabetic hypertensive rats

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Abstract

Angiotensin-(1–7) [Ang-(1–7)] is a vasodilator peptide with cardiac and vascular protective properties. We examined the influence of Ang-(1–7), both endogenous and after chronic treatment with the peptide (576 µg/(kg day)), on ischemia/reperfusion (I/R)-induced cardiac dysfunction in streptozotocin-treated spontaneously hypertensive rats (diabetic SHR). In isolated perfused hearts, recovery of left ventricular function from 40 min of global ischemia was improved significantly in Ang-(1–7)- or captopril-treated diabetic SHR and worsened in animals treated with A779, an Ang-(1–7) receptor (AT_(1–7)) antagonist. The beneficial effect of captopril on cardiac recovery was reduced when co-administered with A779. Cardiac NF-κB activity appears to be higher in diabetic SHR and treatment with Ang-(1–7) or captopril decreased NF-κB activity in diabetic SHR, an effect partially reversed by co-administration of A779. Real-time PCR-based gene array analysis of cardiac tissue revealed that Ang-(1–7) or captopril treatment may reduce expression of several genes of inflammation involved in the NF-κB signalling pathway. The data provide for the first time a role for endogenous Ang-(1–7) as well as confirmation that exogenous treatment with the peptide produces cardioprotection. Whether potential anti-inflammatory and transcriptional factor changes are directly linked to the cardioprotection produced by Ang-(1–7) in diabetic SHR remains to be determined.

Keywords

Hypertension; Diabetes; Heart; Ischemia; NF-κB; Inflammation

1. Introduction

Angiotensin-(1–7) [Ang-(1–7)] is a vasodilator peptide with anti-hypertensive properties [1–4]. The effects of Ang-(1–7) are mediated by an angiotensin receptor selective for Ang-(1–7) [AT_(1–7)] that stimulates the release of vasodilatory prostaglandins and nitric oxide [1,2]. Ang-(1–7) is formed in the heart from Ang I or II by several endopeptidases and carboxypeptidases, including angiotensin-converting enzyme-2 (ACE2) [5–7]. Several studies show that treatment with Ang-(1–7) produces cardioprotective effects [3,4,8,9]. These include reduction of cyclooxygenase-2 expression in cardiac fibroblasts [10]. During ACE inhibition or angiotensin type-1 (AT₁) blockade, Ang-(1–7) plasma levels and cardiac ACE2 are elevated

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and, therefore, part of the beneficial effects of ACE inhibitors and AT₁ blockers may be mediated through Ang-(1–7) [4]. We recently showed that chronic treatment with exogenous Ang-(1–7) produces cardiac protection in terms of recovery from ischemia-reperfusion (I/R) injury in animals with severe hypertension as well as in normotensive diabetic animals [3, 11].

The present study was designed to determine whether treatment with Ang-(1–7) prevents cardiac dysfunction in streptozotocin-treated spontaneously hypertensive rats (diabetic SHR), and whether treatment with A779, an AT_(1–7) receptor antagonist, reveals evidence for Ang-(1–7) as a compensatory endogenous mechanism or a contributor to the cardioprotective effects of captopril. The effect of Ang-(1–7) on cardiac inflammatory responses through the NF-κB signalling pathway was also investigated as a potential mechanism for the protection. Together the results show for the first time that endogenous Ang-(1–7) or chronic elevation of the peptide produces cardioprotection in response to acute I/R injury, associated with inhibition of NF-κB activity and suppression of the expression of inflammatory response genes in diabetic SHR.

2. Methods

2.1. Experimental procedures

Male WKY and SHR weighing about 300 g were used in eight groups ($N = 12/\text{group}$): Group 1 was vehicle-treated WKY; Group 2 was vehicle-treated SHR; Group 3 was Ang-(1–7) (576 μg/(kg day) i.p.)-treated SHR. Group 4 was STZ-treated SHR (diabetic SHR); Group 5 was Ang-(1–7)-treated diabetic SHR; Group 6 was A779 (744 μg/(kg day) i.p.)-treated diabetic SHR; Group 7 was captopril-treated diabetic SHR and Group 8 was captopril + A779-treated diabetic SHR. Animals were euthanized at the end of the 4-week treatment period. Ang-(1–7), A779 and captopril were obtained from Sigma Biochemical (USA).

Diabetes was induced using established protocols by a single i.p. injection of 55 mg/kg body weight STZ dissolved in citrate buffer (pH 4.5), which produces a rapid and sustained reduction in insulin persistent diabetic state. Blood glucose levels were determined using an automated blood glucose analyser (glucometer Elite XL). Body weight was determined after 4 weeks just before sacrificing the animals. All analyses were performed by investigators who were blinded to the treatment groups. The 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 was 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.

2.2. Blood pressure measurement

A separate group of animals were used for measurement of mean arterial pressure (MAP). Animals were anesthetized with sodium pentobarbital (60 mg/kg) and the left femoral artery was exposed surgically at the end of the 4-week period. A small incision was made in the femoral artery, and a catheter was inserted and connected to a pressure transducer for blood pressure measurement. MAP was recorded on a polygraph and expressed as mmHg.

2.3. Heart perfusion studies

At the end of the 4-week period, rats were anesthetized with intraval sodium (40 mg/kg body weight) and hearts were rapidly removed after intravenous heparinization (1000 U/kg body weight). The excised hearts were immediately mounted on the Langendorff perfusion assembly (ML870B2 Langendorff System, ADI Instruments, USA), and were perfused initially with a constant pressure perfusion of 50 mmHg with oxygenated (95% O₂ + 5% CO₂) KH buffer (37 °C). A water-filled balloon was introduced into the left ventricle and connected to a Statham

pressure transducer (P23Db) and balloon volume was adjusted to give the baseline end-diastolic pressure of 5 mmHg. Left ventricular developed pressure (Pmax) and left ventricular end-diastolic pressure (LVEDP) were continuously monitored. Coronary flow (CF) coronary vascular resistance (CVR) were measured by means of an electromagnetic flow probe positioned in the inflow tubing immediately above the aortic per-fusion cannula. Perfusion pressure was measured immediately downstream from the flow probe in a branch of the aortic cannula using a Statham pressure transducer and was electronically maintained constant at 50 mmHg by means of a perfusion pressure control module. This system permits accurate adjustment of perfusion pressure between 5 and 300 mmHg to an accuracy of ± 1 mmHg. Hearts were perfused for 30 min and then subjected to 40 min of ischemia (I) followed by a 30 min period of reperfusion (R). Post-I/R left ventricular contractility and hemodynamics were recorded and compared.

2.4. Preparation of nuclear protein extracts

Nuclear protein extracts from rat hearts subjected to I/R were prepared by a modification of the method of Dignam [12]. The heart tissues taken at the end of the I/R period were homogenized in 5 ml of buffer A (10 mM HEPES; pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, and 0.5 mM DTT) with a Dounce homogenizer. The homogenate was centrifuged at 6000 rpm for 15 min and the pellet was resuspended in 3 ml of extraction buffer (20 mM HEPES; pH 7.9, 25% glycerol, 0.55 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, and protease inhibitor complete (Boehringer Mannheim GmbH, Mannheim, Germany) and homogenized. The extract was centrifuged in a micro-centrifuge at maximal speed for 30 min, and the supernatant was dialyzed against 1 l of dialysis buffer (40 mM KCl, 15 mM HEPES; pH 7.9, 1 mM EDTA, 0.5 mM PMSF, 0.5 mM DTT, and 20% glycerol) for 2 h. The extract was centrifuged in a micro-centrifuge for 10 min, and the supernatant was stored at $-80^\circ C$ prior to use. The concentration of total proteins in the samples was determined by the Pierce protein assay reagent (Pierce Chemical, Rockford, IL, USA).

2.5. NF- κ B binding activity

A colorimetric assay was performed to measure the NF- κ B activity using a 96-well-enzyme linked immunosorbent assay (ELISA) (Chemicon International, USA). This assay has been validated to provide quantification of NF- κ B activity in numerous studies (10, 28, 34, 41). In a 50- μ l binding reaction mixture containing binding buffer [50 μ g/ml of double-stranded poly (dI-dC), 10 mM Tris HCl (pH 7.5), 50 mM NaCl, 0.5 mM EDTA, 0.5 mM DTT, 1 mM $MgCl_2$, and 10% glycerol], 15 μ g of nuclear proteins extract, and 2 pmole of biotinylated double stranded oligonucleotide probe (1 pmol/ μ l) containing the wild-type consensus sequence (5'-GGGACTTTC-3') for NF κ B. The binding reaction mixture was incubated at room temperature for 30 min and the bound NF κ B transcription factor subunits p65 was detected with specific primary antibodies, a rabbit anti-NF κ B p65. A highly sensitive HRP-conjugated secondary antibody was then used for detection. The absorbance of the samples using a spectrophotometric microplate reader set at $\lambda 450$ nm. Specific NF- κ B binding activity to its DNA element was confirmed by the addition of non-labelled NF- κ B specific competitor that abolished observed absorbance at $\lambda 450$ nm.

2.6. Gene array analysis

NF- κ B gene array analysis was performed in the heart tissue taken at the end of I/R study using the NF- κ B Signalling Pathway real-time PCR-based Array (SuperArray, Frederick, MD, USA). Each array is a 96-well plate that includes primer sets for 84 NF- κ B signalling pathway genes plus five housekeeping genes and two negative controls. Briefly, RNA was isolated from the hearts of all animals in each group and reverse transcribed into first strand cDNA using ReactionReady™ First Strand cDNA Synthesis Kit (Super-Array, Frederick, MD, USA). The

cDNA was then amplified using a PCR master mix and aliquoted into each well of the 96-well plate containing pre-dispensed gene-specific primer sets. Real-time detection of PCR amplicons was enabled using SYBR green included in the PCR master mix. Relative gene expression was determined using an ABI Prism 7000 real-time thermal cycler (Applied Biosystems, Foster City, CA, USA). The changes in gene expression of each gene were calculated from the mean of the Ct values for both control and target genes obtained from duplicate reaction runs using the $2^{-\Delta C_t}$ method.

2.7. Statistical analysis

Differences were considered to be significant when p value was less than 0.05. Data are presented as mean $\pm$ SEM of 'n' number of experiments. Heart perfusion results were compared with their respective baseline controls using a two tailed, paired t -test. Comparison between different experimental groups was done by a general factorial analysis of variance. Computerized statistical analysis was accomplished with SPSS for Windows (V.6.0.1; SPSS Inc., Evanston, IL, USA). Further comparisons were made by obtaining univariate Scheffes' confidence intervals for the parametric estimates. NF- κ B activity results were analyzed using GraphPad Prism software. Mean values were compared using analysis of variance followed by post hoc tests (Bonferroni).

3. Results

3.1. Hyperglycemia and MAP

Induction of diabetes by STZ resulted in a significant increase in blood glucose concentration. Hyperglycemia persisted in the diabetic SHR and was 520 ± 58 mg/dl at 4 weeks as compared with 89 ± 3 mg/dl in WKY or 92 ± 5 mg/dl in SHR. Blood glucose concentration at the end of the 4-week treatment period was not significantly altered by any of the treatment strategies. In agreement with previous observations [21], induction of diabetes with STZ resulted in significant attenuation of MAP in SHR (SHR: 205 ± 11 mmHg vs. diabetic SHR: 163 ± 3 mmHg). Treatment of diabetic SHR with Ang-(1-7) did not cause a significant change in MAP (164 ± 8 mmHg).

3.2. Cardiac recovery from ischemia/reperfusion

Tables 1 and 2 provide the actual values for Pmax, LVEDP, coronary flow and coronary vascular resistance. Recovery of cardiac function was worse in SHR and diabetic SHR compared to WKY, consistent with previous reports [21]. Treatment with Ang-(1-7) improved recovery in both SHR and diabetic SHR. In diabetic SHR, captopril-induced improvement in recovery was attenuated when captopril was given in combination with A779. Treatment with A779 alone resulted in worsened recovery.

3.3. Ang-(1-7) regulates cardiac NF- κ B activity

The relative optical density (OD) reflecting the NF- κ B binding activity to its DNA element is presented in Table 3. NF- κ B activity was higher in SHR as compared to WKY and also higher in diabetic SHR as compared to SHR. Treatment with Ang-(1-7) decreased NF- κ B activity in both SHR and diabetic SHR whereas treatment with A779 tended to increase NF- κ B activity. The captopril-induced decrease in NF- κ B activity was partially prevented when captopril was given in combination with A779.

3.4. Activation of NF κ B signalling pathway

An NF- κ B target real-time PCR gene array was used to determine whether Ang-(1-7) treatment caused a decrease of NF- κ B target gene expression in concert with the changes in the NF- κ B binding activity. Of the 84 genes on the NF- κ B targeted real-time PCR gene array, although

many exhibited changes, either positive or negative, only genes with ≥ 2 -fold change are shown in Table 4. Significant gene expression changes were obtained for genes directly involved in the regulation of the NF- κ B signalling cascade leading to the activation of the inflammatory response pathway. Ang-(1–7) treatment down regulated key genes responsible for the activation of the NF- κ B pathway including toll-like receptor 2 (Tlr2), interleukin-1 receptor-associated kinase 1 (Irak1) and inhibitor of kappaB kinase beta (Ikkb). NF- κ B downstream effector pro-inflammatory genes [complement component 3 (C3), interleukin-1 beta (Il-1 β), interleukin-6 (Il-6), (NACHT-containing protein) Nalp12 and caspase 1 (Casp1)] were also markedly reduced by Ang-(1–7) or captopril in SHR and diabetic SHR models.

While Ang-(1–7) had similar qualitative effects in diabetic and non-diabetic SHR, Ang-(1–7)-induced decrease in expression of inflammatory genes was more pronounced in diabetic SHR.

4. Discussion

Chronic treatment with Ang-(1–7) or captopril attenuated I/R-induced cardiac dysfunction in SHR and diabetic SHR. Similar protective effects of Ang-(1–7) are reported in WKY and diabetic WKY previously [11]. Therefore, Ang-(1–7) clearly provides cardioprotective effects during global cardiac ischemia independent of either diabetes or hypertension. An important new finding, revealed by blockade of Ang-(1–7) receptors indicates that endogenous Ang-(1–7) contributes to cardioprotection during I/R of diabetic SHR and participates in the effect of captopril-induced improvement in cardiac recovery. Thus, the magnitude of I/R injury in an individual may depend upon endogenous Ang-(1–7) generation. Novel findings further include that treatment with Ang-(1–7) may decrease NF- κ B activity, receptors and signalling as well as genes of inflammation in both SHR and diabetic SHR, whereas treatment with the AT_(1–7) antagonist, A779, may increase or prevent partially the captopril-induced decreases. These latter findings are consistent with numerous previous reports that diabetes and hypertension activate NF- κ B and inflammation and that ACE inhibitors and AT₁ receptor blockers decrease these pathways [13–16].

Ang II is one of the main agents involved in the development of hypertension- and/or diabetes-induced end organ damage. ACE inhibitors and AT₁ blockers are effective in reducing NF- κ B activity, Ang II-induced inflammation and end-organ damage in models of hypertension and diabetes [13,16]. During ACE inhibition or AT₁ blockade, Ang-(1–7) plasma levels and cardiac ACE2 are elevated, and the beneficial effects of ACE inhibitors and AT₁ blockers appear to be mediated in part through Ang-(1–7) [4,17]. The results of the present study show that Ang-(1–7), both endogenous and exogenously administered, and captopril produced similar cardioprotective effects in diabetic SHR, and that captopril-induced cardioprotection was reduced when captopril was given in combination with A779. These observations suggest that long term treatment with Ang-(1–7) in patients with diabetes and hypertension could be effective in subsequent prevention of cardiac dysfunction in the face of ischemia. Cardioprotective effects of captopril are mediated in part through Ang-(1–7), since co-administration of A779 resulted in significant decrease in captopril-induced improvement in cardiac function. However, the actions of Ang-(1–7) are independent of any blood pressure lowering effect, since diabetic SHR have lower blood pressure than control SHR and this is not further reduced by Ang-(1–7) [18].

The mechanisms involved in Ang-(1–7)-mediated cardiovascular protection are not well characterized. The beneficial effects of Ang-(1–7) in the cardiovascular system are mediated by prostaglandin and nitric oxide which have been shown to be protective against I/R-induced dysfunction [19–21]. Ang-(1–7) has been shown to have direct protective effects on cardiac muscle by preventing generation of cardiac arrhythmias during I/R [22]. Ang-(1–7)-mediated improved recovery observed in this study is probably due to both improved hemodynamics

and reduced generation of arrhythmias. The data from the present study indicate that Ang-(1–7) treatment as well as the endogenous peptide, resulted in reduction of cardiac dysfunction in response to I/R in association with reduced NF- κ B activity and reduced expression of upstream and downstream associated pathways in the hearts of SHR and diabetic SHR. Although we have not established a direct causal link among these events, the ELISA-based enzyme activity and the real-time PCR-based gene array yield complementary data to show that endogenous Ang-(1–7) may reduce key factors involved in activation of genes of inflammation. The present findings extend the potential mechanisms for the actions of Ang-(1–7) to limit inflammation, since others have shown that treatment with Ang-(1–7) reduces cyclooxygenase-2 expression in isolated cardiac fibroblasts [10].

Ang-(1–7) may also act indirectly since chronic infusion of Ang-(1–7) results in decreased cardiac Ang II levels [23]. Thus, Ang-(1–7)-induced cardioprotection may be at least partly mediated by reduced Ang II signalling. It is well established that Ang II-induced inflammation contributes to end-organ damage in hypertension and/or diabetes and are elicited by activation of the NF- κ B canonical pathway [24,25]. In this study, we demonstrate increased activity of NF- κ B and elevated expression of the toll signalling pathway in SHR and diabetic SHR. Nuclear factor- κ B (NF- κ B) is a key transcription factor that regulates inflammatory, stress, proliferative and apoptotic responses to a large number of stimuli. In the cardiovascular system, NF- κ B is associated with myocarditis, I/R injury, myocardial infarction, heart failure, cardiac hypertrophy, and atherosclerosis [15,26]. NF- κ B is activated in the heart by tumor necrosis factor- α (TNF- α), G protein-coupled agonists, and by oxidative stress leading to the transcription of proinflammatory cytokines, adhesion molecules and reactive oxygen species (ROS) generating enzymes [15,26]. NF- κ B may be involved in the development of hypertension- and diabetes-induced end-organ damage [14,25]. In animal models of hypertension, increased NF- κ B activity has been demonstrated in angiotensin II (Ang II)-induced inflammatory responses via angiotensin type-1 (AT₁) receptors [24]. Inhibition of NF- κ B activity has been shown to be cardioprotective against I/R injury [25]. The blockade of the renin-angiotensin system with angiotensin converting enzyme (ACE) inhibitors or AT₁ receptor blockers decrease NF- κ B, inflammation and cardiovascular damage [13]. Chronic treatment with Ang-(1–7) prevented activation of Tlr2, Ikbkb and Irak1, and reduced NF- κ B activity in SHR and diabetic SHR indicating that inhibition of this pathway may contribute to the cardioprotective effects of Ang-(1–7) in hypertension and/or diabetes. Ang-(1–7)-induced inactivation of NF- κ B also led to the down regulation of several proinflammatory cytokines and mediators in both SHR and diabetic SHR animals. Down regulation of the expression of C3, Il-6, Il-1 β , Nalp12 and Casp1 by Ang-(1–7) suggests that Ang-(1–7)-mediated cardioprotection is at least partly a result of reduced activity of these pro-inflammatory mediators. A recent study by Higuchi et al. showed in mice overexpressing TNF- α that cardioprotection afforded by NF- κ B ablation is associated with activation of Akt [26]. Ang-(1–7) has been shown to stimulate endothelial NO synthase activation and NO production via Akt-dependent pathways in human aortic endothelial cells [27] suggesting that the cardioprotective effects of Ang-(1–7) observed in this study may be at least partly a result of activation of signalling involving Akt.

The question of what cells within the heart are responsible for the beneficial responses to Ang-(1–7) remains to be addressed. Ang-(1–7) exhibits actions on isolated myocytes and fibroblasts [10]. In intact heart, Ang-(1–7) is known to provide anti-arrhythmic and anti-fibrotic effects [8,28]. Endothelial cells and macrophages in coronary arteries from ischemic hearts and to a lesser extent from nonischemic cardiomyopathic hearts contribute to inflammation [29]. Ang-(1–7) receptors are on vascular elements [30,31]. During ischemia, Ang-(1–7) reduces the impact of superoxide to negate the effect of nitric oxide on platelets [32]. The response to I/R injury and actions of Ang-(1–7) in our study likely involve interactions among these various cell types.

In conclusion, our study demonstrated that endogenous Ang-(1–7) may act to limit cardiac dysfunction in diabetes and hypertension. Long term treatment with exogenously administered Ang-(1–7) was effective in attenuating cardiac dysfunction in response to acute I/R and this may be associated with anti-inflammatory actions. While a definitive link between the cardioprotection and the suppression of inflammation is possible, further studies are required. This does not exclude additional actions of the peptide to improve the response of the heart to I/R. Therapies that elevate Ang-(1–7), such as ACE inhibitors, also appear to owe at least part of their beneficial effects to the endogenous elevation of this peptide.

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Table 1
Effect of Ang-(1-7) on post-ischemic recovery in global contractility.

Groups studied	Pmax (mmHg)			LVEDP (mmHg)		
	Control	Reperfusion	%R	Control	Reperfusion	%R
1. WKY	79 ± 6	42 ± 4	52 ± 3	7.2 ± 0.3	25 ± 3	346 ± 33
2. SHR	117 ± 7	36 ± 4	31 ± 4 [*]	7.8 ± 0.5	39 ± 3	501 ± 54 [*]
3. SHR-Ang-(1-7)	116 ± 5	49 ± 2	42 ± 2 ^{*,†}	6.0 ± 0.3	25 ± 2	410 ± 34 [†]
4. Diabetic SHR	57 ± 9	7 ± 2	11 ± 3 ^{*,†}	6.8 ± 0.3	56 ± 5	833 ± 87 ^{*,†}
5. Diabetic SHR + Ang-(1-7)	69 ± 6	13 ± 2	19 ± 2 ^{*,†,§}	6.5 ± 0.2	46 ± 4	698 ± 53 ^{*,†,§}
6. Diabetic SHR + A779	38 ± 5	3 ± 2	7 ± 3 ^{*,†}	5.8 ± 0.2	66 ± 3	1149 ± 91 ^{*,†,§}
7. Diabetic SHR + captopril	71 ± 6	17 ± 2	24 ± 2 ^{*,§}	6.7 ± 0.2	45 ± 2	665 ± 13 ^{*,†,§}
8. Diabetic SHR + captopril + A779	42 ± 6	7 ± 2	16 ± 4 ^{*,#}	5.7 ± 0.2	54 ± 3	950 ± 46 ^{*,†,#}

The data were computed at baseline (control) and again after 40 min of ischemia and a 30-min reperfusion period, and expressed as mean ± SEM (N = 6). Pmax = left ventricular developed pressure; LVEDP = left ventricular end-diastolic pressure; %R = % recovery (reperfusion/control).

^{*} Value significantly different from WKY, $p < 0.05$.

[†] Value significantly different from SHR, $p < 0.05$.

[§] Value significantly different from diabetic SHR, $p < 0.05$.

[#] Value significantly different from diabetic SHR-captopril, $p < 0.05$.

Table
2 Effect of Ang-(1-7) on post-ischemic recovery in hemodynamics.

Groups studied	Coronary flow (ml min ⁻¹)			Coronary vascular resistance (mmHg ml ⁻¹ min ⁻¹)		
	Control	Reperfusion	%R	Control	Reperfusion	%R
1. WKY	10.2 ± 0.5	4.5 ± 0.5	44 ± 4	4.4 ± 0.4	21 ± 2	486 ± 19
2. SHR	8.0 ± 0.3	2.0 ± 0.4	25 ± 5 [*]	6.8 ± 0.4	43 ± 5	627 ± 48 [*]
3. SHR-Ang-(1-7)	10.5 ± 0.4	3.4 ± 0.2	33 ± 2 ^{*,†}	5.5 ± 0.3	28 ± 2	529 ± 40 [†]
4. Diabetic SHR	5.5 ± 0.4	0.8 ± 0.2	14 ± 3 ^{*,†}	9.0 ± 0.4	66 ± 3	739 ± 18 ^{*,†}
5. Diabetic SHR + Ang-(1-7)	6.1 ± 0.1	1.3 ± 0.2	22 ± 3 ^{*,§}	8.7 ± 0.2	60 ± 1	694 ± 13 ^{*,§}
6. Diabetic SHR + A779	5.0 ± 0.3	0.2 ± 0.1	4 ± 2 ^{*,†,§}	14.0 ± 1.2	88 ± 4	789 ± 19 ^{*,†,§}
7. Diabetic SHR + captopril	7.2 ± 0.3	1.5 ± 0.1	21 ± 2 ^{*,§}	7.2 ± 0.3	47 ± 4	656 ± 22 ^{*,§}
8. Diabetic SHR + captopril + A779	5.3 ± 0.5	0.9 ± 0.2	16 ± 2 ^{*,†,#}	9.8 ± 0.5	72 ± 3	739 ± 10 ^{*,†,#}

The data were computed at baseline (control) and again after 40 min of ischemia and a 30-min reperfusion period, and expressed as mean ± SEM (N = 6). %R = % recovery (reperfusion/control).

^{*} Value significantly different from WKY, $p < 0.05$.

[†] Value significantly different from SHR, $p < 0.05$.

[§] Value significantly different from diabetic SHR, $p < 0.05$.

[#] Value significantly different from diabetic SHR-captopril, $p < 0.05$.

Table 3

Effect of Ang-(1–7) on NF-κB DNA-binding activity.

Groups studied	NF-κB activity
1. WKY	7.0 ± 0.5
2. SHR	25.0 ± 0.9 [*]
3. SHR-Ang-(1–7)	3.6 ± 0.3 ^{*,†}
4. Diabetic SHR	33.5 ± 1.3 ^{*,†}
5. Diabetic SHR-Ang-(1–7)	3.5 ± 0.4 ^{*,†,§}
6. Diabetic SHR-A779	38.7 ± 2.3 ^{*,†,§}
7. Diabetic SHR-captopril	10.5 ± 0.6 ^{*,†,§}
8. Diabetic SHR-captopril-A779	21.6 ± 0.8 ^{*,†,§,#}

The data are expressed as mean ± SEM ($N = 5$).

^{*} Value significantly different from WKY, $p < 0.05$.

[†] Value significantly different from SHR, $p < 0.05$.

[§] Value significantly different from diabetic SHR, $p < 0.05$.

[#] Value significantly different from diabetic SHR-captopril, $p < 0.05$.

Table 4Change in gene expression in the NF- κ B signalling pathway.

	WKY	SHR	SHR-Ang-(1-7)	Diabetic SHR	Diabetic SHR+Ang-(1-7)	Diabetic SHR+captopril
Activation of the NF- κ B pathway						
NM_198769 Trl2	1	1.52	0.99	1.52	0.88	0.83
NM_053355 Ikbb/AIM-1	1	1.58	0.87	1.92	0.66	0.64
XM_343844 Irak1	1	2.01	1.03	2.35	0.69	0.77
Inflammatory response genes						
NM_016994 C3	1	2.45	0.70	2.85	0.66	0.63
NM_031512 Il-1 β	1	1.85	1.21	2.68	0.54	0.64
NM_012589 Il-6	1	1.30	0.92	2.58	0.52	0.61
XM_218181 Nalp12	1	2.60	1.40	2.09	0.26	0.33
NM_012762 Casp1	1	2.45	0.90	3.16	0.55	0.68

Reactions were run in duplicates using an RNA sample pooled from three rats. Changes in gene expression were calculated based on the $2^{-\Delta C_t}$ method. All the values indicate the increase (above 1) or decrease (below 1) compared to WKY.