Angiotensin-(1–7) prevents diabetes-induced cardiovascular dysfunction

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Benter IF, Yousif MH, Cojocel C, Al-Maghrebi M, Diz DI. Angiotensin-(1–7) prevents diabetes-induced cardiovascular dysfunction. Am J Physiol Heart Circ Physiol 292: H666–H672, 2007; doi:10.1152/ajpheart.00372.2006.—The aim of this study was to test the hypothesis that treatment with angiotensin-(1–7) [ANG-(1–7)] or ANG-(1–7) nonpeptide analog AVE-0991 can produce protection against diabetes-induced cardiovascular dysfunction. We examined the influence of chronic treatment (4 wk) with ANG-(1–7) (576 μg·kg−1·day−1 ip) or AVE-0991 (576 μg·kg−1·day−1 ip) on proteinuria, vascular responsiveness of isolated carotid and renal artery ring segments and mesenteric bed to vasoactive agonists, and cardiac recovery from ischemia-reperfusion in streptozotocin-treated rats (diabetes). Animals were killed 4 wk after induction of diabetes and/or treatment with ANG-(1–7) or AVE-0991. There was a significant increase in urine protein (231 ± 2 mg/24 h) in diabetic animals compared with controls (88 ± 6 mg/24 h). Treatment of diabetic animals with ANG-(1–7) or AVE-0991 resulted in a significant reduction in urine protein compared with vehicle-treated diabetic animals (183 ± 16 and 149 ± 15 mg/24 h, respectively). Treatment with ANG-(1–7) or AVE-0991 also prevented the diabetes-induced abnormal vascular responsiveness to norepinephrine, endothelin-1, angiotensin II, carbachol, and histamine in the perfused mesenteric bed and isolated carotid and renal arteries. In isolated perfused hearts, recovery of left ventricular function from 40 min of global ischemia was significantly better in ANG-(1–7)- or AVE-0991-treated animals. These results suggest that activation of ANG-(1–7)-mediated signal transduction could be an important therapeutic strategy to reduce cardiovascular events in diabetic patients.

DIABETES MELLITUS IS A MAJOR debilitating disease affecting millions worldwide. The quality of life of patients with diabetes is largely determined by the complications rather than the primary disease. Among these, micro- and macrovascular dysfunctions are probably the most dominant factors because they result in a three- to fivefold increase in deaths in diabetics compared with the normal population. Diabetes-induced cardiovascular dysfunction is evidenced clinically by accelerated atherosclerosis, retinopathy, nephropathy, occlusive vascular disease, and hypertension (18, 26, 34). Alterations within the renin-angiotensin system are considered to be important for the development of diabetic complications, particularly diabetic renal disease and hypertension (10, 38, 46, 47). Suppression of angiotensin II (ANG II) synthesis or activity can prevent or slow the progression of diabetes-induced cardiovascular complications. Indeed, angiotensin-converting enzyme (ACE) inhibitors and ANG II receptor blockers have become an integral part of any therapeutic strategy to reduce renal and cardiovascular events in patients with diabetes (10, 35).

Angiotensin-(1–7) [ANG-(1–7)] is a vasodilator peptide shown to have antihypertensive (3, 4, 6), antithrombotic and antiproliferative properties (20, 27, 28, 44, 45, 49). The effects of ANG-(1–7) are mediated through the G protein-coupled receptor mas (39) and involve activation of vasodilatory prostaglandins and nitric oxide (NO) (3, 4, 11, 16, 39). ANG-(1–7) is formed from ANG I and II by several endopeptidases and carboxypeptidases and metabolized by ACE (3, 4, 11, 16, 17, 30, 50). ANG-(1–7) plasma levels are elevated during ACE inhibition or ANG II type I receptor (AT1) blockade. In fact, the effects of ACE inhibitors and AT1 blockers may be mediated, at least in part, through ANG-(1–7) (16, 21–24, 36). Blockade of AT1 receptors in spontaneously hypertensive rats (SHR) results in increased expression of ACE2 and ANG-(1–7) production and in pressure-independent prevention of vascular remodeling of vasculature (19). Chronic treatment with ANG-(1–7) provides blood pressure lowering and improvements in renal, cardiac, and vascular function similar to many of the beneficial effects of renin-angiotensin system blockade in a model of hypertension with reduced NO synthesis (6). A recent study showed that there is an increase in renal ACE2 protein and a decrease in ACE protein in diabetic (db/db) mice, suggesting that the pattern of low ACE protein coupled with increased ACE2 protein expression may be renoprotective in diabetes (48). The present study was designed to determine whether chronic treatment with ANG-(1–7) or ANG-(1–7) nonpeptide analog AVE-0991 can attenuate development of cardiovascular dysfunction in a model of streptozotocin (STZ)-induced diabetes.

MATERIALS AND METHODS

Experimental Procedures

Male Wistar rats weighing about 300 g were used in this study. The rats had free access to food and water throughout the study. Animals were divided into six groups. Group I (control) was vehicle-treated animals. Groups II and III were control animals treated with daily injections of ANG-(1–7) (576 μg·kg−1·day−1) or AVE-0991 (576 μg·kg−1·day−1), respectively. Group IV (diabetic; D) was STZ-treated animals. Groups V [D + ANG-(1–7)] and VI (D + AVE-0991) were diabetic rats treated with ANG-(1–7) or AVE-0991, respectively. Animals were killed at the end of the 4-wk treatment period.

Diabetes was induced by a single intraperitoneal injection of 55 mg/kg body wt STZ dissolved in citrate buffer (pH 4.5). Age-matched control rats were injected with the citrate buffer vehicle used to dissolve STZ. Body weight and basal glucose levels were determined

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before STZ injection with the use of an automated blood glucose analyzer (glucometer Elite XL). Blood glucose concentrations were determined 48 h after STZ injection. Rats with a blood glucose concentration above 200 mg/dl were declared diabetic. The animals’ body weight and diabetic state were reassessed after 4 wk immediately before the animals were killed. The dose of the ANG-(1–7) was chosen on the basis of our previous studies in models of hypertension (4, 6). 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.

Urine Volume and Protein Determination

At the end of the 4-wk treatment period, animals were placed in metabolic cages, and food and water were provided ad libitum. Metabolic cages provided an effective separation of feces and urine into tubes outside the cage. The urine collection was carried out for 24 h. During this period, tubes used for urine collection were immersed in an ice-cold water bath to avoid loss in enzyme activity. Total protein and lysozyme concentration in the urine were determined as described previously (6, 12, 41).

Vascular Reactivity Experiments

Vascular reactivity of the mesenteric bed and renal and carotid arteries to vasoactive agents was studied as described by us previously (5, 7). Briefly, the mesenteric artery was cannulated and perfused with Krebs-Henseleit (KH) solution (at 37°C), oxygenated with 95% O2-5% CO2, delivered at a constant flow rate of 6 ml/min using a multichannel masterflex peristaltic pump. The composition of KH solution was as follows (in mM): 118.3 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 MgSO4, 25 NaHCO3, 1.2 KH2PO4, and 11.2 glucose. Changes in perfusion pressure that reflect peripheral resistance were measured (in mmHg). The isolated carotid and renal arteries were cut into ring segments of about 8- or 5-mm segments, respectively, and mounted in organ baths containing 50 ml KH solution at pH 7.4. The tissue bath solution was maintained at 37°C and was aerated with 95% O2-5% CO2 mixture. Isometric contractions of the carotid and renal arteries were recorded (5, 7). The vasoactive agonists were chosen on the

Table 1. Effect of ANG-(1–7) and AVE-0991 on body weight and kidney function

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, g</th>
<th>Urine Volume, ml/24 h</th>
<th>Urine Protein, mg/24 h</th>
<th>Urine Lysozyme, μg/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>358 ± 9</td>
<td>7 ± 0.8</td>
<td>88 ± 6</td>
<td>43 ± 5</td>
</tr>
<tr>
<td>Diabetic</td>
<td>223 ± 26*</td>
<td>124 ± 4*</td>
<td>231 ± 2*</td>
<td>459 ± 3*</td>
</tr>
<tr>
<td>D + ANG-(1–7)</td>
<td>329 ± 12†</td>
<td>121 ± 2*</td>
<td>183 ± 16†</td>
<td>454 ± 6*</td>
</tr>
<tr>
<td>D + AVE-0991</td>
<td>256 ± 4*</td>
<td>110 ± 8*</td>
<td>149 ± 15†</td>
<td>424 ± 31*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5–8 experiments. ANG-(1–7), angiotensin-(1–7)-treated diabetic group; D + ANG-(1–7), AVE-0991-treated diabetic group. *Value significantly different from control, P < 0.05; †value significantly different from diabetes, P < 0.05.

Fig. 1. A: norepinephrine (NE)-induced vasoconstriction (10, 100 and 1,000 nmol) in the perfused mesenteric vascular bed of control (C), angiotensin-(1–7) [ANG-(1–7)]-treated control [C + ANG-(1–7)], AVE-0991-treated control (C + AVE-0991), diabetic (D), ANG-(1–7)-treated diabetic [D + ANG-(1–7)], and AVE-0991-treated diabetic (D + AVE-0991) animals. B: endothelin-1-induced vasoconstriction (0.1 and 1.0 nmol) in the perfused mesenteric vascular bed of control, C + ANG-(1–7), C + AVE-0991, diabetic, D + ANG-(1–7), and D + AVE-0991 animals. C: carbachol-induced vasodilation (1.0 and 10 nmol) in the perfused mesenteric vascular bed of control, C + ANG-(1–7), C + AVE-0991, diabetic, D + ANG-(1–7), and D + AVE-0991 animals precontracted with NE (10–5 M). Values are means ± SE; n = 6–8. *Significantly different compared with control animals; †significantly different compared with the diabetic animals.
basis of their potential role in regulating blood pressure. The vasocostrictor agonists [norepinephrine (NE), ANG II, and endothelin-1 (ET-1)] are potent vasocostrictor agonists that are endogenously released in various vascular beds and have a potential role in the maintenance of blood pressure and vascular resistance. The vasodilator agonists (carbachol and histamine) are endothelium-dependent agonists. Response to sodium nitroprusside (SNP), which is an endothelium-independent agonist, indicates the integrity of the underlying smooth muscle.

Vasoconstriction Studies

Following the period of equilibration, the vasoconstrictor responses of NE (10, 100, and 1,000 nmol) or ET-1 (0.01, 0.1, and 1.0 nmol) were investigated in the perfused mesenteric vascular bed. Successive doses of the agonists NE or ET-1 were given at regular intervals to establish the vasoconstrictor responses (in mmHg) (7). The vasocostrictor effects of NE (10^{-8}, 10^{-7}, and 10^{-6} M), ET-1 (10^{-10}, 10^{-9}, and 10^{-8} M), or ANG II (10^{-9}, 10^{-8}, and 10^{-7} M) were tested in the isolated segments of the carotid or renal artery. The different concentrations of the agonists were added successively to the organ baths to establish the vasoconstrictor responses (5).

Vasodilation Studies

Following the period of equilibration, the vasodilator responses of carbachol, histamine, and SNP were investigated in the perfused mesenteric vascular bed. The perfused mesenteric bed was constricted by perfusion with KH solution containing NE (10^{-5} M). After a steady level of precontraction was established, successive doses of carbachol (1 and 10 nmol), histamine (10 and 100 nmol), or SNP (0.1, 1.0, and 10 nmol) were given at regular intervals (7). The vasodilator response is expressed as the percentage of the precontraction induced by NE (10^{-5} M). The isolated segments of carotid or renal artery were precontracted by a submaximal concentration of NE (10^{-6} M) added to the organ baths. After a steady level of contraction was obtained, the vasodilator effects of carbachol (10^{-7}, 10^{-6}, and 10^{-5} M), histamine (10^{-6}, 10^{-5}, and 10^{-4} M), or SNP (10^{-8}, 3 × 10^{-8}, and 10^{-7} M) were tested. The vasodilator response was measured as the percent change in the maximal initial contraction induced by NE (10^{-6} M) (5).

Heart Perfusion Studies

Rats were anesthetized with thiopental sodium (Intraval Sodium, 40 mg/kg body wt), and hearts were rapidly removed after intravenous heparinization (1,000 U/kg body wt). The excised hearts were immediately mounted on the Langendorff perfusion assembly (model ML870B2 Langendorff System, ADI Instruments) and were perfused initially with a constant pressure perfusion of 50 mmHg with oxygenated (95% O_{2}-5% CO_{2}) KH buffer (37 °C). A water-filled balloon was introduced into the left ventricle and connected to a Statham pressure transducer (model P23Db), and balloon volume was adjusted to give the baseline end-diastolic pressure of 5 mmHg. Left ventricular developed pressure (P_{max}) was continuously monitored. Coronary flow was measured bymeans of an electromagnetic flow probe positioned in the inflow tubing immediately above the aortic perfusion 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 followed by a period of 30 min reperfusion (I/R). Post-I/R left ventricular contractility and hemodynamics were recorded and compared as described by us previously (6).

Statistical Analysis

Urine volume and protein and vascular reactivity results were analyzed with the use of GraphPad Prism software. Data are presented as means ± SE of n number of experiments. Mean values were compared using analysis of variance followed by post hoc tests (Bonferroni). The difference was considered to be significant when P < 0.05. Heart perfusion results are expressed as means ± SE.
Reperfusion values 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 (version 6.0.1; SPSS, Evanston, IL). Further comparisons were made by obtaining univariate Scheffe’s confidence intervals for the parametric estimates.

**Drugs**

dl-NE-bitartrate, STZ, ANG-(1–7), ANG II, histamine, SNP, ET-1, and carbachol were obtained from Sigma Biochemical. AVE-0991 was a gift from Aventis.

**RESULTS**

**Hyperglycemia and Animal Body Weight**

Induction of diabetes by STZ resulted in a significant increase in blood glucose concentration. Hyperglycemia persisted in the diabetic animals and was 570 ± 18 mg/dl at 4 wk as compared with 91 ± 3 mg/dl in the control animals. There was a significant reduction in the weight of STZ-diabetic rats (223 ± 26 g) compared with the nondiabetic control animals (358 ± 9 g) (P < 0.05). Blood glucose concentration was not corrected by 4-wk treatment of the STZ-diabetic animals with either ANG-(1–7) or AVE-0991. However, chronic treatment of diabetic animals with ANG-(1–7) resulted in prevention of body weight loss compared with vehicle-treated diabetic animals (P < 0.05) (Table 1).

**Urine Volume and Protein**

STZ treatment resulted in a significant increase in urine volume (124 ± 4 ml/24 h) and urine protein (231 ± 2 mg/24 h) as compared with controls (Table 1). Treatment of diabetic animals with ANG-(1–7) or AVE-0991 resulted in a significant reduction in urine protein compared with vehicle-treated diabetic animals (P < 0.05) (Table 1).

**Vascular Reactivity Results**

**Mesenteric bed.** The response to NE, ET-1, and carbachol was not affected in the mesenteric bed of control animals treated with ANG-(1–7) or AVE-0991 compared with the control vehicle-treated rats. The vasoconstrictor response to ET-1 and ANG II was significantly augmented in the isolated mesenteric arteries of diabetic animals compared with controls (Fig. 1, A and B). Treatment of diabetic animals with ANG-(1–7) resulted in a significant attenuation of the vasoconstrictor responses to NE (10 and 100 nmol) and ET-1 (0.1 and 1.0 nmol) compared with vehicle-treated diabetic animals (P < 0.05) (Fig. 1, A and B). AVE-0991 was more effective in reversing NE than ET responses. The reduced vasodilator response to low-dose (1 nmol) carbachol in the perfused mesenteric vascular bed from diabetic animals compared with vehicle-treated diabetic animals was corrected with ANG-(1–7) or AVE-0991 treatment (Fig. 1C). The vasodilator response to SNP (0.1, 1.0, and 10 nmol) was similar in all the groups studied (data not shown).

**Carotid artery.** The response to ET-1, ANG II, carbachol, and histamine was not affected in the carotid artery of control animals treated with ANG-(1–7) or AVE-0991 compared with the vehicle-treated controls. NE (100 and 1,000 nmol)- and ET-1 (0.1 nmol)-induced vasoconstrictor responses were significantly augmented (P < 0.05) in the perfused mesenteric beds from diabetic animals compared with controls (Fig. 1, A and B). Treatment of diabetic animals with ANG-(1–7) resulted in a significant attenuation of the vasoconstrictor responses to ET-1 and ANG II compared with vehicle-treated diabetic animals (P < 0.05) (Fig. 1, A and B). AVE-0991 was more effective in reversing NE than ET responses. The reduced vasodilator response to low-dose (1 nmol) carbachol in the perfused mesenteric vascular bed from diabetic animals compared with vehicle-treated diabetic animals was corrected with ANG-(1–7) or AVE-0991 treatment (Fig. 1C). The vasodilator response to SNP (0.1, 1.0, and 10 nmol) was similar in all the groups studied (data not shown).

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diabetic animals ($P < 0.05$) (Fig. 2, A and B). The vasodilator responses to carbachol ($10^{-6}$ and $10^{-5}$ M) and histamine ($10^{-5}$ and $10^{-4}$ M) were significantly reduced in the isolated carotid artery of diabetic animals (Fig. 2, C and D). Treatment of the diabetic animals with ANG-(1–7) or AVE-0991 resulted in a significant increase in the vasodilator responses to carbachol and histamine compared with vehicle-treated diabetic animals ($P < 0.05$) (Fig. 2, C and D). The vasodilator responses to SNP ($10^{-6}$ and $3 \times 10^{-8}$ M) were similar in all the groups studied (data not shown).

**Renal artery.** The response to NE, ET-1, ANG II, carbachol, and histamine was not affected in the renal artery of control animals treated with ANG-(1–7) or AVE-0991 compared with the control vehicle-treated rats. The vasoconstrictor responses to NE (10 and 100 nM), ET-1 (1 nM), and ANG II (1, 10, and 100 nM) were significantly potentiated in the isolated renal artery of diabetic animals (Fig. 3, A and B). Chronic treatment of diabetic animals with ANG-(1–7) or AVE-0991 resulted in a significant attenuation of the vasoconstrictor responses to NE, ET-1, and ANG II in the isolated renal artery compared with vehicle-treated diabetic animals ($P < 0.05$) (Fig. 3, A and B). The vasodilator responses to carbachol ($10^{-7}$, $10^{-6}$, and $10^{-5}$ M), histamine ($10^{-6}$, $3 \times 10^{-5}$, and $10^{-4}$ M) or SNP ($10^{-8}$, $3 \times 10^{-8}$, and $10^{-7}$ M) were not affected in the renal artery of the diabetic rats compared with the control nondiabetic rats. Carbachol-induced vasodilation ($10^{-4}$ M) was tested in all the preparations and confirmed the integrity of the endothelium.

**Cardiac Recovery From I/R**

Table 2 provides the actual values for $P_{max}$ and coronary flow. Treatment with ANG-(1–7) or AVE-0991 improved recovery in cardiac function in hearts from diabetic animals to the level of hearts from control animals.

**DISCUSSION**

The major finding of this study was that chronic ANG-(1–7) treatment resulted in a significant protection against diabetes-induced cardiovascular dysfunction. The beneficial effects of chronic ANG-(1–7) treatment in diabetic animals included attenuation of proteinuria, protection of the heart in response to global I/R, and restoration of normal reactivity to constrictor and dilator stimuli in the vasculature. A similar action was observed with ANG-(1–7) nonpeptide analog AVE-0991.

ANG II overactivity is thought to play a pivotal role in the development of diabetic nephropathy (10, 38, 46). The glomerular hypertension responsible for diabetic nephropathy results from an interaction between preglomerular capillary vasodilatation provoked by hyperglycemia and postglomerular ANGII-mediated vasoconstriction (18, 29, 38, 46). Suppression of ANG II synthesis or activity can prevent or slow the progression of diabetic nephropathy (10, 26). Indeed, ACE inhibitors and angiotensin receptor blockers are preferred drugs in the treatment of hypertension-associated with diabetes and proteinuria (10, 38). Results from this study have shown that diabetes resulted in significant increase in proteinuria, a marker of renal glomerular injury. Treatment with ANG-(1–7) or AVE-0991 significantly attenuated diabetes-induced proteinuria. ANG II effects in the kidney microvasculature are predominantly reduced by NO, and proteinuria is a result of unchecked actions of ANG II on the glomerular circulation in the absence of this key modulator (10, 46). Thus the mechanism of protection by either ANG-(1–7) or AVE-0991 could be due to stimulation of the release of vasodilatory prostaglandins and NO (6). These results are in agreement with the observation that there is an increase in renal ACE2-to-ACE protein ratio in diabetic (db/db) mice, suggesting that reduced ANG II accumulation and increased ANG-(1–7) formation may be renoprotective in diabetes (48). Interestingly, treatment of diabetic rats with an ACE inhibitor, which results in elevated ANG-(1–7) levels, produces a similar increase in renal ACE2-to-ACE protein ratio (30). There is growing evidence that tubular injury is a major feature in the development of renal dysfunction in diabetes (51). Low-molecular-weight proteins such as lysozyme are recognized as markers of tubular dysfunction (51). The results of the present study indicate that ANG-(1–7) and AVE-0991 provide protection only against glomerular injury but not against tubular damage.

ANG-(1–7) can be generated locally within the myocardium because ACE2 is expressed in the heart (17, 50). Infusion of ANG-(1–7) in rats after myocardial infarction improves cardiac function and attenuates the development of heart failure (1, 32). ANG-(1–7), which can reduce the growth of cardiomyocytes through activation of the mas receptor, has also been shown to have antifibrotic and antitrophic effects in cardiac fibroblasts (20, 45). It has been shown that myocardial infarction results in increased expression of ACE2 in rat and humans, and intravenous infusion of ANG-(1–7) lasting for 8 wk and commencing 2 wk after induction of myocardial infarction in rats induced a marked regression of left ventricular failure (17, 32). In the present study, recovery of left ventricular function from 40 min of global ischemia was significantly impaired in isolated perfused hearts from diabetic animals compared with control animals. Hearts from ANG-(1–7)- or AVE-0991-treated diabetic rats recovered from ischemia, with $P_{max}$ and coronary flow values similar to that of control animals. The cardioprotection provided by ANG-(1–7) occurred in the presence of continued hyperglycemia. Thus restoring prostaglan-

### Table 2. Effect of ANG-(1–7) and AVE-0991 on postischemic recovery in global contractility and hemodynamics

<table>
<thead>
<tr>
<th>Group</th>
<th>Perfusion Developed Pressure, mmHg</th>
<th>Reperfusion</th>
<th>%R</th>
<th>Coronary Flow, ml/min</th>
<th>Reperfusion</th>
<th>%R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>51 ± 9</td>
<td>19 ± 3</td>
<td>39 ± 4</td>
<td>6.6 ± 0.6</td>
<td>2.1 ± 0.3</td>
<td>32 ± 5</td>
</tr>
<tr>
<td>Diabetic</td>
<td>86 ± 14</td>
<td>24 ± 9</td>
<td>28 ± 4*</td>
<td>8.5 ± 0.6</td>
<td>1.5 ± 0.2</td>
<td>18 ± 4*</td>
</tr>
<tr>
<td>D + ANG-(1–7)</td>
<td>115 ± 12</td>
<td>44 ± 3</td>
<td>42 ± 7†</td>
<td>9.5 ± 0.5</td>
<td>3.4 ± 0.3</td>
<td>36 ± 3†</td>
</tr>
<tr>
<td>D + AVE-0991</td>
<td>94 ± 10</td>
<td>48 ± 8</td>
<td>51 ± 8†</td>
<td>7.9 ± 0.6</td>
<td>3.1 ± 0.4</td>
<td>41 ± 4†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 experiments. The data were computed at 30-min reperfusion. %R, %recovery (reperfusion/control). *Value significantly different from control, $P < 0.05$; †value significantly different from diabetes, $P < 0.05$.
dins and NO and providing protection from fibrosis by ANG-(1–7) or AVE-0991 may contribute to overall improvements in vascular and cardiac health, similar to what occurs in the case of ACE inhibitor treatments, despite the fact that hyperglycemia is not corrected.

Diabetes mellitus is associated with alterations in vascular reactivity that contributes to the development of serious cardiovascular complications (13, 15, 25, 33, 40). Enhanced vascular responsiveness to vasoconstrictors and attenuated responses to vasodilators have been reported in diabetic patients and animals (2, 15, 25, 33, 37). However, the factors contributing to the changes in vascular reactivity in diabetes are not fully understood. Endothelial dysfunction is a common characteristic of diabetes (9, 15), and hyperglycemia results in diminished release and/or bioavailability of nitric oxide following administration of endothelial-dependent dilators (2, 25, 37, 43). Thus impaired NO synthesis may be partially responsible for the impaired effects of vasoconstrictors and vasodilators observed in diabetic animals. In this study, we found that treatment with ANG-(1–7) or AVE-0991 led to correction of the altered vasoconstrictor responses to NE or ET-1 and vasodilator response to carbachol in the mesenteric bed of diabetic rats, indicating that activation of ANG-(1–7) receptors can produce protection against development of diabetes-induced vascular dysfunction. ANG-(1–7) or AVE-0991 treatment also attenuated the diabetes-induced vascular changes revealed by responses to ET-1, ANG II, carbachol, and histamine in the carotid artery. In the renal artery from diabetic rats, vasodilation in response to carbachol, histamine, and SNP was not significantly different in the various groups studied. However, the exaggerated response to vasoconstrictor agonists NE, ET-1, and ANG II observed in the renal artery of diabetic rats was attenuated by treatment with ANG-(1–7) or AVE-0991.

Whereas current experiments did not attempt to investigate the precise mechanisms involved in the overall effects of ANG-(1–7), it is well known that actions of this peptide include release of vasodilatory prostaglandins and NO and potentiation of the actions of and release or protection of kinins (3, 4, 8, 31). ANG-(1–7) releases prostacyclin, which has autocrine and paracrine effects on vascular prostacyclin receptors to increase cAMP, activate the cAMP-dependent protein kinase, and reduce mitogen-activated protein kinase activities to inhibit vascular growth (44). Exogenous ANG-(1–7) inhibited vascular smooth muscle cell proliferation associated with balloon-catheter injury (42). It has been suggested that ANG-(1–7) treatment also plays a role in attenuation of neointimal formation by structural recovery of the endothelium (14, 31). Previous data using an ANG-(1–7) treatment protocol comparable to the present study revealed similar correction of vascular impairments in SHR treated with Nω-nitro-l-arginine methyl ester (6). These observations support the hypothesis that activation of ANG-(1–7)-mediated signaling can be an effective strategy to prevent diabetes-induced or other vascular dysfunction associated with reduced NO availability. The lack of effect of ANG-(1–7) or AVE-0991 on the responses in the nondiabetic control rats is consistent with previous data showing that infusion of the peptide in the absence of hypertension does not lower pressure (4). These findings suggest that perhaps a deficiency of ANG-(1–7) is a potential mechanism for the diabetic-induced changes and that replacement of the peptide reverses this aspect of the pathology.

In summary, we showed that ANG-(1–7) and its analog AVE-0991 can attenuate the development of diabetes-induced cardiovascular dysfunction in the absence of correcting hyperglycemia. This implies that, although certain signaling pathways may be triggered by hyperglycemia/diabetes, their subsequent blockade via other mechanisms can, at least partially, prevent end-organ dysfunction. We conclude that activation of ANG-(1–7)-mediated signal transduction pathway appears to be an important therapeutic strategy to reduce cardiovascular events in diabetic patients and may underlie a portion of the beneficial effects of ACE inhibition or AT1 blockade.

GRANTS
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ANG-(1–7) PREVENTS CARDIOVASCULAR DYSFUNCTION IN DIABETES