ELSEVIER

European Journal of Pharmacology 365 (1999) 225–232

ejp

Cardiovascular effects of captopril and enalapril in obese Zucker rats

Juan Duarte *, Andres Martinez, Almudena Bermejo, Beatriz Vera, Maria José Gámez, Paloma Cabo, Antonio Zarzuelo

Department of Pharmacology, School of Pharmacy, University of Granada, 18071 Granada, Spain

Received 23 July 1998; revised 19 November 1998; accepted 24 November 1998

Abstract

The effects of two weeks of oral administration of the angiotensin-converting enzyme inhibitors captopril (a sulphydryl-containing drug) and enalapril (which lacks the sulphydryl group) on skeletal muscle glucose uptake, arterial blood pressure, cardiac hypertrophy, proteinuria and aortic vascular reactivity in obese Zucker rats were evaluated. Captopril (50 mg kg^{-1} once daily) and enalapril (10 mg kg^{-1}) did not modify body weight gain or food or water intake. Both drugs decreased systolic blood pressure (157 ± 6, 133 ± 4 and 136 ± 3 mm Hg, in vehicle-, captopril- and enalapril-treated rats, respectively), blood glucose (172 ± 8 vs. 151 ± 7 and 158 ± 5 mg dl^{-1}, respectively), proteinuria (46 ± 10 vs. 17 ± 2 and 18 ± 2.5 mg dl^{-1}, respectively) and heart weight (2.17 ± 0.03, 1.98 ± 0.02 and 1.99 ± 0.04 mg g^{-1} of body weight, respectively). Plasma insulin concentration was significantly increased by enalapril (17 ± 2 ng ml^{-1} vs. 9 ± 2) but not by captopril (12 ± 1). In the absence of insulin, the diaphragms from captopril- or enalapril-treated rats showed a significantly higher glucose uptake than that of controls (31% and 30% vs. control group, respectively). The presence of insulin in the incubation medium did not stimulate peripheral glucose uptake in the control group but significantly increased glucose uptake in diaphragms from captopril- or enalapril-treated rats (enhancement of glucose uptake vs. control: 52% and 43%, respectively). Endothelium-intact aortic rings from control Zucker rats showed a poor relaxant response to acetylcholine (maximal relaxation of $38.4 \pm 4.7\%$). Captopril significantly improved the endothelium-dependent vascular relaxation responses to acetylcholine and the endothelium-independent relaxation to the nitric oxide donor sodium nitroprusside whereas enalapril did not modify these relaxant responses. Neither captopril nor enalapril significantly affected the vascular contractile responses to the vasoconstrictors noradrenaline or KCl. In conclusion, the angiotensin-converting enzyme inhibitors captopril and enalapril reversed insulin resistance and the associated cardiovascular complications (cardiac hypertrophy, hypertension and proteinuria) in the obese Zucker rat, an animal model of non-insulin-dependent (type II) diabetes mellitus. However, only captopril, but not enalapril, improved the impaired endothelium-dependent and independent relaxant responses in the isolated rat aorta.

Keywords: Captopril; Enalapril; Insulin resistance; Cardiac hypertrophy; Aorta; (Zucker rat)

1. Introduction

Insulin resistance is a common feature of several disorders such as obesity, non-insulin-dependent diabetes mellitus and hypertension (Ferrannini et al., 1987). In both human and animal studies, insulin resistance has been associated with cardiovascular complications including increased blood pressure, cardiac and vascular hypertrophy, coronary heart disease and stroke (Modan et al., 1985; Reaven, 1988). It has been proposed that the management of insulin resistance may contribute to the prevention of cardiovascular events (Kaplan, 1989). Angiotensin-converting enzyme inhibitors have a beneficial effect on insulin resistance besides their blood pressure-lowering effect, suggesting a role for angiotensin II in the mechanism of insulin resistance (Lithell, 1991; Uehara et al., 1994; Henriksen and Jacob, 1995). Furthermore, Angiotensin-converting enzyme inhibitors have been shown to prevent the cardiovascular complications associated with hypertension and insulin resistance in both human and animals studies (Dzau, 1989; Linz et al., 1995; Waeber and Brunner, 1996).

The genetically obese Zucker rat (fa/fa) is a widely used model of non-insulin-dependent (type II) diabetes mellitus, characterized by hyperinsulinemia, obesity and hyperlipidemia in association with glucose intolerance (Bray, 1977; Ionescu et al., 1985; King et al., 1992). The

* Corresponding author. Tel.: +34-58-243889; Fax: +34-58-248964; E-mail: jmduarte@platon.ugr.es

0014-2999/99/$ - see front matter
PII: S0014-2999(98)00879-6

association of hypertension with diabetes in this model appears to be inconsistent: some researchers found no difference in blood pressure between obese Zucker rats and their lean counterparts (Levin et al., 1984; Pawloski et al., 1992) while others found a higher blood pressure in the obese Zucker rat (Kurtz et al., 1989; Zemel et al., 1992). Changes in vascular responsiveness to vasoconstrictors and vasodilators have also been reported in this model, in both in vivo (Zemel et al., 1992) and in vitro experiments (Zemel et al., 1990, 1991; Cox and Kikta, 1992; Harker et al., 1993; Wu et al., 1996; Walker et al., 1997). Both acute and chronic administration of angiotensin-converting enzyme inhibitors significantly enhanced in vitro insulin-mediated muscle glucose uptake activity in Zucker rats (Henriksen and Jacob, 1995; Henriksen et al., 1996).

To our knowledge, the prevention of the cardiovascular events (hypertension, impaired vascular reactivity, cardiac hypertrophy and proteinuria) concomitant with insulin resistance in the obese Zucker rat has not yet been determined. Therefore, the aim of the present study was to evaluate the effects of a two-week treatment with angiotensin-converting enzyme inhibitors on skeletal muscle glucose uptake, arterial blood pressure, cardiac hypertrophy and vascular reactivity. Currently available angiotensin-converting enzyme inhibitors can be divided into two categories: those with a sulphydryl group and those without a sulphydryl group. Thus, we compared the effects of captopril (a sulphydryl-containing angiotensin-converting enzyme inhibitor) and enalapril (an angiotensin-converting enzyme inhibitor lacking the sulphydryl group).

2. Methods

2.1. Experimental animals and blood pressure measurement

Ten-week-old, female obese Zucker rats (Harlan Iberica, Barcelona, Spain) were used in the study. The rats were kept in groups of two in plastic cages (23 × 55 cm) at a room temperature of 22–23°C with a controlled light/dark cycle (12/12 h). The arrival of the purchased animals was followed by an adaptation period of two weeks before the initiation of the experiment. Ten animals were assigned to each of the three treatment groups that consisted of control, captopril and enalapril. The animals received by gavage one daily dose (10 a.m.) of captopril (50 mg kg^{-1} body weight), enalapril (10 mg kg^{-1} body weight) or vehicle (distilled water) for two weeks. We chose a dose of captopril that in a previous report (Henriksen and Jacob, 1995) improved glucose uptake in skeletal muscle in Zucker rats. Furthermore, this dose of captopril is between a dose that produces acute hypotensive effect in spontaneously hypertensive rats (SHR) (10 mg kg^{-1} i.v., Cachofeiro et al., 1992) and the maximal effective dose to decrease blood pressure in SHR (100 mg kg^{-1} day^{-1} orally during 2 weeks, Clozel et al., 1990). The choice of the dose of enalapril was done on the basis of equieffectivity as antihypertensive (a dose of enalapril five times lower than that of captopril, Creager and Roddy, 1994). During this experimental period the rats had free access to tap water and chow. Systolic blood pressure was measured using tail-cuff plethysmography at the beginning of the experiment and just before killing. At least seven determinations (at 10 p.m.) were made in every session and the mean of the lowest three values within 5 mm Hg was taken as the systolic blood pressure. Body weight and systolic blood pressure were measured at the beginning and at the end of the experiment. The consumption of chow and tap water was measured by weighing the chow and water bottles, respectively.

At the end of the experimental period the animals were killed and exsanguinated after they had been deprived of food overnight. No drug or vehicle was administered the last day so that only long-term effects of the drugs could be observed. Proteinuria was determined by a semiquantitative colorimetric method (Albym-test, Boehringer Mannheim, East Sussex, UK). Blood samples were taken for glucose and insulin measurement. Blood glucose was measured by enzymatic colorimetric methods (Linear Chemicals, Badalona, Spain). Serum insulin was determined by radioimmunoassay (Biotrak, Amersham Iberica, Madrid, Spain). The heart was excised, cleaned and weighed. The heart wet weight-to-body weight ratio was calculated as an index of cardiac hypertrophy.

2.2. Peripheral glucose uptake

Glucose uptake was studied in rat diaphragm preparations. Diaphragms were divided into two halves and incubated in a 3-ml chamber at 37.2°C, with constant oxygenation for 90 min and shaking at 90 cycles/min as previously described (Vallance-Owen, 1972; Zarzuelo et al., 1996). The bathing solution was prepared as follows: 125 ml 1.3% $NaHCO_3$ was aerated for 3 min with a 95% O_2–5% CO_2 mixture and was then added to 750 ml of saline solution. The resultant mixture was aerated for 10

Table 1
Values of body weight, heart weight and metabolic variables of Zucker rat after treatment for two weeks with different drugs

	Controls	Captopril	Enalapril
Initial body weight (g)	335 ± 7	325 ± 10	341 ± 7
Final body weight (g)	381 ± 9	371 ± 10	390 ± 9
Heart wet weight (mg)	830 ± 27	734 ± 21[b]	783 ± 23[a]
Food intake (g day^{-1})	21.8 ± 0.3	20.4 ± 0.4	23.2 ± 0.3
Water intake (ml day^{-1})	29.6 ± 2.1	37.4 ± 2.0	28.6 ± 1.6
Blood glucose (mg dl^{-1})	171.8 ± 8.0	150.6 ± 7.0[a]	157.7 ± 5.0
Plasma insulin (ng ml^{-1})	9.5 ± 1.8	12.2 ± 1.4	17.4 ± 2.1[b]
Proteinuria (mg dl^{-1})	46.0 ± 9.7	17.0 ± 2.1[b]	18.0 ± 2.5[b]

Data are expressed as means ± S.E.M.; $n = 10$ in each group.
[a] $P < 0.05$ and [b] $P < 0.01$ vs. control group.

min and used immediately. The composition of the saline solution (in mM) was NaCl 139.3, KCl 5.86, $NaHCO_3$ 25.71, $MgSO_4$ 1.22, $CaCl_2$ 2.31, KH_2PO_4 1.26 and glucose 16. One hemidiaphragm from each animal was incubated in the presence of insulin (2 mU ml^{-1}). The results are expressed as glucose uptake (by subtracting glucose uptake after incubation from glucose uptake before incubation) per 10 mg dry diaphragm. Dry weight was determined after oven drying the material at 105°C for 120 min.

2.3. Aortic responses in vitro

After the rats were killed, the descending thoracic aortas were quickly dissected and placed in a modified physiological saline solution (PSS) of the following composition (mM): NaCl 118, KCl 4.75, $NaHCO_3$ 25, $MgSO_4$ 1.2, $CaCl_2$ 2, KH_2PO_4 1.2 and glucose 11. The aorta was cleaned of fat and connective tissue and cut into 3-mm rings. Two L-shaped stainless-steel wires were inserted into the arterial lumen and the rings were placed in Allhin organ chambers filled with PSS at 37°C and gassed with 95% O_2 and 5% CO_2. One wire was attached to the chamber and the other to an isometric force-displacement transducer (Letigraph 2000, Letica) as previously described (Duarte et al., 1994). The rings were stretched to a resting tension of 2 g and allowed to equilibrate for 60–90 min. During this period tissues were re-stretched and washed every 30 min with warm PSS. After equilibration, concentration–contractile response curves for noradrenaline (10^{-9} M–10^{-5} M) or KCl (8 mM–80 mM) were made by increasing the organ chamber concentration by cumulative increments after a steady-state response had been reached with each increment. The concentration–relaxation response curves for acetylcholine (10^{-8} M–10^{-5} M) and sodium nitroprusside (10^{-10} M–10^{-5} M) were determined in aortas pre-contracted with noradrenaline 10^{-6} M. Only one agonist was tested in each aortic ring.

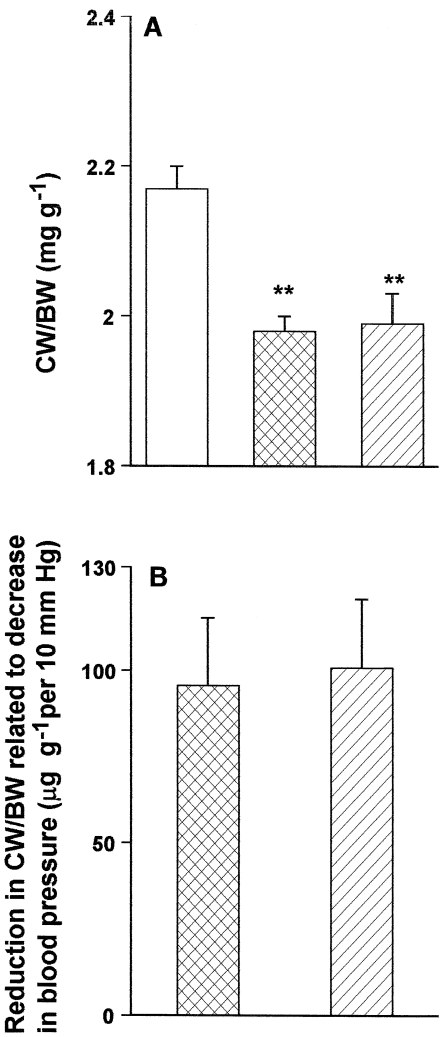

Fig. 1. Effects of captopril and enalapril on cardiac hypertrophy. (A) Cardiac hypertrophy expressed as cardiac weight (CW) to body weight (BW) ratio (CW/BW). Control (open bars), captopril (crosshatched bars), enalapril (hatched bars). (B) Reduction in cardiac weight related to the drug-induced decrease in systolic blood pressure, expressed as CW/BW per 10 mm Hg, of Zucker rats. Captopril (crosshatched bars), enalapril (hatched bars). Data are expressed as means ± S.E.M. of 10 experiments. $**P < 0.01$ vs. control group.

Table 2
Effect of captopril and enalapril treatment on in vitro skeletal muscle glucose uptake in the absence or in the presence of insulin (2 mU/ml)

	Control	Captopril	Enalapril
Without insulin	8.7 ± 0.4	11.4 ± 0.7[b]	11.3 ± 0.4[b]
With insulin	9.4 ± 0.4	14.3 ± 0.6[b(c)]	13.4 ± 0.4[b(c)]

The results are expressed as concentration of glucose uptake (mg/100 ml) per 10 mg dry diaphragm. Each value represents the mean ± S.E.M. ($n = 10$).
[b] $P < 0.01$ vs. control group.
[(c)] $P < 0.01$ vs. without insulin incubation.

2.4. Drugs

The following drugs were used: captopril (Squibb Industria Farmaceutica, Barcelona, Spain), acetylcholine chloride, enalapril maleate, (±)-noradrenaline bitartrate, porcine insulin and sodium nitroprusside (Sigma, St. Louis, MO, USA). All drugs were dissolved in distilled deionized water. Ascorbic acid (10^{-4} M) was added to the stock solution of noradrenaline to prevent oxidation. Further dilutions were made in Krebs solution.

2.5. Statistical analysis

Results are expressed as means ± S.E.M. Cumulative concentration–response curves determined in each ring were fitted to the logistic equation $y = E_{max}/(1 + \exp(-K(x + pD_2))$, where the E_{max} represents the maximal effect induced by the agonist, K the slope of the curve and pD_2 the negative logarithm of the concentration of agonist producing 50% of the E_{max}. Statistically significant differ-

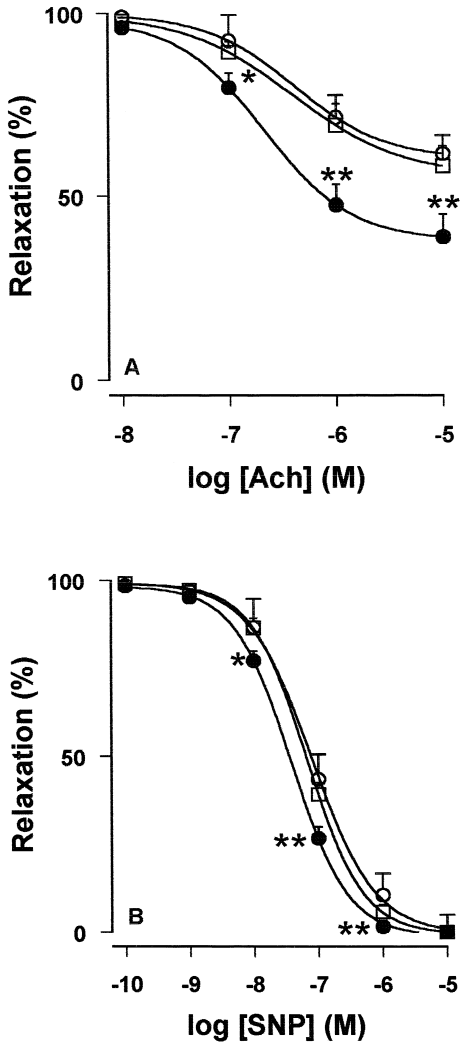

Fig. 2. Relaxant responses to acetylcholine (Ach) (A) and sodium nitroprusside (SNP) (B) in isolated endothelium-intact aortic rings precontracted with noradrenaline (1 μM) from Zucker rats after two weeks on different drug regimens: (○) control group; (●) captopril group; (□) enalapril group. Data are expressed as means ± S.E.M. of 10 experiments in each group.

ences were calculated by means of an analysis of variance followed by a Newman–Keuls test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Blood pressure, cardiac hypertrophy and metabolic variables

In the three groups, systolic blood pressure values at the beginning of the experiment were in the range 140–150 mm Hg. After two weeks of treatment, both captopril and enalapril decreased systolic blood pressure significantly by 15.7% and 13.6% vs. control group, respectively. No statistically significant differences in final body weight, food intake and water intake were found. However, blood glucose, proteinuria and heart weight were reduced in the captopril and enalapril groups (Table 1). Plasma insulin was significantly increased (83% vs. control group) by enalapril but the increase was not significant for captopril. Captopril and enalapril decreased the cardiac hypertrophy index by 8.7% and 8.3%, respectively (Fig. 1a). When related to the drug-induced reduction in blood pressure, the effect on the cardiac hypertrophy index was similar for both treatments (Fig. 1b).

3.2. Diaphragm glucose uptake

In the absence of insulin, the diaphragms from captopril- or enalapril-treated rats showed significantly a higher glucose uptake than control diaphragms (31% and 30% vs. control group, respectively). The presence of insulin in the incubation medium did not modify peripheral glucose uptake in control diaphragms, indicating a resistance to the action of insulin in Zucker rats. However, insulin significantly increased glucose uptake in diaphragms from captopril- or enalapril-treated rats (increase in glucose uptake vs. control: 52% and 43%, respectively) (Table 2). Thus, the treatment with enalapril and captopril reversed insulin resistance.

3.3. Vascular reactivity

Endothelium intact aortic rings from control Zucker rats showed a poor relaxant response to acetylcholine (maximal relaxation of 38.4 ± 4.7%). However, captopril (but not enalapril) significantly improved the endothelium-dependent vascular relaxation responses to acetylcholine (Fig. 2), increasing both the maximal relaxation and the pD_2 value (Table 3). Furthermore, aortic rings from captopril-treated rats also showed enhanced endothelium-indepen-

Table 3
Parameters of contractile and relaxant responses of isolated aortic rings from Zucker rats after two weeks of treatment with different drugs

	Control	Captopril	Enalapril
Relaxation (pD_2)			
Acetylcholine	6.42 ± 0.07	6.75 ± 0.08[b]	6.54 ± 0.11
Sodium nitroprusside	7.10 ± 0.07	7.45 ± 0.08[b]	7.21 ± 0.08
Maximal relaxation (%)			
Acetylcholine	38.4 ± 4.7	61.0 ± 6.0[b]	41.8 ± 5.3
Sodium nitroprusside	100.0 ± 0.3	100.5 ± 1.0	99.3 ± 0.6
Contraction (pD_2)			
Noradrenaline	7.71 ± 0.10	7.75 ± 0.16	7.87 ± 0.18
KCl	1.65 ± 0.03	1.63 ± 0.05	1.67 ± 0.05
Maximal force (g)			
Noradrenaline	5.15 ± 0.24	4.85 ± 0.19	4.67 ± 0.27
KCl	3.12 ± 0.30	3.10 ± 0.21	2.89 ± 0.27

Maximal relaxations are expressed as percentages of 1 μM of noradrenaline-induced precontraction. Values are means ± S.E.M. ($n = 10$) in each group.
[b] $P < 0.01$ vs. control group.

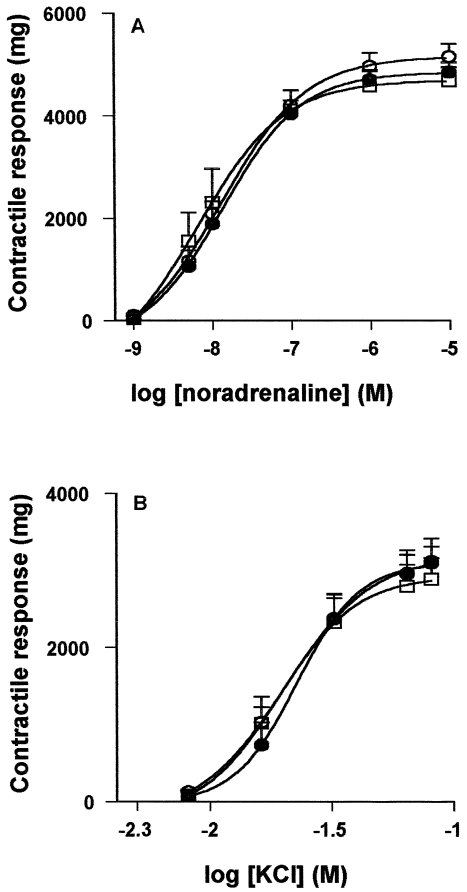

Fig. 3. Contractile responses to noradrenaline (A) and to potassium chloride (KCl) (B) of isolated endothelium-intact aortic rings from Zucker rats after two weeks on different drug regimens: (○) control group; (●) captopril group; (□) enalapril group. Data are expressed as means ± S.E.M. of 10 experiments in each group.

dent relaxation in response to the nitric oxide donor sodium nitroprusside whereas enalapril did not modify these relaxant responses. In contrast, neither captopril nor enalapril significantly affected the vascular contractile responses to the vasoconstrictors noradrenaline or KCl (Fig. 3, Table 3).

4. Discussion

In the present study we demonstrated that oral administration for two weeks of captopril or enalapril increased insulin-stimulated glucose uptake in skeletal muscle from obese Zucker rats. This effect was accompanied by a reduction in systolic blood pressure and a decrease in cardiac mass and reduced proteinuria. Furthermore, only captopril, a sulphydryl-containing drug, increased the nitric oxide-mediated vascular relaxation in response to acetylcholine and sodium nitroprusside in rat isolated aorta.

The renin–angiotensin system is recognized as a key factor in blood pressure regulation and electrolyte homeostasis. The renin–angiotensin system constitutes a proteolytic cascade in which angiotensinogen from the liver is cleaved by renin to produce a decapeptide, angiotensin I. Angiotensin I is converted to the octapeptide, angiotensin II, by the angiotensin-converting enzyme (Smith et al., 1992), a nonspecific protease which is also responsible for the degradation of bradykinin as well as peptides such substance P and enkephalins (Erdoes and Skidgel, 1986). Therefore, the effects of angiotensin-converting enzyme inhibitors are not only mediated by a decreased synthesis of angiotensin II but also by their potentiation of the effects of bradykinin.

Resistance to the insulin-induced stimulation of glucose transport has been a consistent finding in studies using the obese Zucker rat, whether assessed by glucose uptake over the entire hindlimb (Kemmer et al., 1979) or by transport of glucose analogs into individual muscles during hindlimb perfusion (Sherman et al., 1988) or in isolated-muscle preparations: soleus muscle (Crettaz et al., 1980), epitrochlearis muscle (Henriksen and Jacob, 1995). Our findings for the diaphragm, an isolated muscle preparation widely used to study glucose uptake, are consistent with these studies. In diaphragms from captopril- or enalapril-treated rats, the ability of insulin to increase glucose uptake was restored, i.e., these drugs reversed insulin resistance. Furthermore, long-term captopril or enalapril treatment stimulated glucose uptake in muscles incubated without insulin, indicating that the beneficial effects of these agents are not restricted to the insulin pathway. These similar findings for captopril and enalapril are consistent with the interpretation that this effect likely represents a class effect of the angiotensin-converting enzyme inhibitors on the glucose transport system in skeletal muscle. The improvement in insulin sensitivity associated with angiotensin-converting enzyme inhibitors was independent of the sulphydryl group, in contrast to the results of previous studies (Uehara et al., 1994), which reported that angiotensin-converting enzyme inhibitors with a sulphydryl group had a more potent action on the improvement in insulin sensitivity in dogs and humans than those without a sulphydryl group. Long-term captopril or enalapril treatment reduced plasma glucose levels (although the reduction was not significant for enalapril). This effect can be attributed to the increased sensitivity to insulin but also to the drug-induced increase in plasma insulin levels (which was significant for enalapril but not for captopril). It is conceivable that an increase in capillary insulin transport secondary to vasodilatation with increased capillary area is reflected by a greater insulin sensitivity. The insulin resistance of both muscle (present results) and liver has been shown to be reduced by kinins and angiotensin-converting enzyme inhibitors, resulting in a lowering of both blood pressure and blood glucose concentration (Bao et al., 1992). It has been shown, using the highly potent, specific, long-acting bradykinin B_2 receptor antagonist, DArg[Hyp^3,Thi^5,DTic^7,Oic^8] bradykinin (Hoe 140), that the potentiation of endogenous kinins can contribute

to the antihypertensive action of angiotensin-converting enzyme inhibitors, thereby improving glucose metabolism (Bao et al., 1992; Tomiyama et al., 1994). However, the decrease in blood pressure after losartan treatment was not accompanied by a fall in glucose level, which suggests that the beneficial effect on glucose metabolism produced by angiotensin-converting enzyme inhibition depends on bradykinin accumulation and is not related to angiotensin II antagonism (Tomiyama et al., 1994; Rosenthal et al., 1997). However, the involvement of angiotensin II or kinins in the effects of angiotensin-converting enzyme inhibitors in obese Zucker rats requires further investigation.

Discrepancies regarding the association of hypertension and insulin-resistance in Zucker obese rats can be found in the literature. According to some previous reports (Kurtz et al., 1989; Zemel et al., 1992) but in contrast to others (Levin et al., 1984; Pawloski et al., 1992), we found a high systolic blood pressure in Zucker rats. Hypertension in Zucker rats has been related to hyperinsulinemia and insulin resistance (Modan et al., 1985). Insulin may exert biphasic effects on vascular tone, such that there is a concentration-dependent balance between direct vasodilatation and noradrenaline-induced vasoconstriction secondary to increased sympathetic neural output. Also, insulin induces renal sodium retention (Peuler and Mark, 1989; Anderson et al., 1991). After two weeks of treatment with captopril and enalapril we detected a decrease in systolic blood pressure. However, we also found that angiotensin-converting enzyme inhibitors increased plasma insulin levels and improved insulin sensitivity, which demonstrated that the beneficial effects of these agents on blood pressure were independent of the plasma insulin levels.

Arterial hypertension accelerates the progression of diabetic nephropathy by inducing hyperfiltration and glomerular hypertension. Several studies demonstrated that glomerular capillary pressure was not elevated in young (9–14 weeks) obese Zucker rats in which increases in mesangial matrix and urine albumin excretion were already evident (Kasiske et al., 1985; O'Donnell et al., 1985). In the present study, we found an antiproteinuric effect of both angiotensin-converting enzyme inhibitors after two weeks of treatment, which agrees with the results of previous studies with hypertensive type-II diabetic patients with nephropathy (Bochicchio et al., 1991) or with Zucker rats (Schmitz et al., 1992).

High blood pressure is one of the most powerful determinants of the development of cardiac hypertrophy (Frochlich et al., 1993). In the present study, final heart wet weights were lower in the long-term captopril- and enalapril-treated obese animals than in the obese control group, irrespective of whether this weight was expressed in absolute terms or relative to final body weight. This decreased growth of the myocardium in both drug-treated groups is consistent with the known effect of angiotensin-converting enzyme inhibition in reversing left ventricular hypertrophy in hypertension (Linz et al., 1995), and is a further evidence for the positive systemic action of these agents.

An increase in peripheral resistance is a characteristic finding in established hypertension. Peripheral resistance may increase as a result of either enhanced contractility or impaired relaxation of vascular smooth muscle. Endothelial cells modulate the underlying vascular smooth muscle tone by releasing endothelium-derived relaxing factors (EDRF) and endothelium-derived contracting factor (Furchgott, 1983; Furchgott and Vanhoutte, 1989). Few reports have studied vascular reactivity in Zucker rats aortic rings. Equal or increased contractile responses to vasoconstrictors (KCl, noradrenaline) were found in obese rats when compared with those of age-matched lean controls (Zemel et al., 1990, 1991; Harker et al., 1993). In the present study, no change in vascular contractile responses to KCl and noradrenaline were observed after long-term treatment with both angiotensin-converting enzyme inhibitors. Impaired endothelium-dependent relaxation in isolated vessels from Zucker rats has also been reported in most (Wu et al., 1996; Walker et al., 1997) but not all studies (Cox and Kikta, 1992; Bohlen and Lash, 1995). The presence of impaired endothelium-dependent relaxation in both Type I and Type II diabetes has also been postulated (Zemel et al., 1990; Feletou et al., 1994). We found a small relaxant response to acetylcholine in endothelium-intact aortic rings from Zucker rats (maximal relaxation: $38.4 \pm 4.7\%$ of 1 μM noradrenaline-induced contraction) whereas in our experience, the maximal relaxation in response to acetylcholine in aorta from lean (Wistar) rats under the same experimental conditions is always in the range of 60–80%. The most intriguing observation of our study is that captopril, the inhibitor with a sulphydryl group, but not enalapril, improved endothelium-dependent relaxations in Zucker rats. Captopril not only increased the maximal relaxation in response to acetylcholine but also increased the sensitivity to this endothelium-dependent vasodilator. Furthermore, the aortas from captopril-treated rats were also more sensitive to the relaxant effect of nitroprusside, an NO donor. Therefore, the increased endothelium-dependent relaxation induced by captopril can be explained, at least partly, by an increased relaxant effect of nitric oxide, rather than by an increased release of nitric oxide. Angiotensin-converting enzyme inhibitors with a sulphydryl moiety would be particularly likely to enhance nitric oxide-induced vasodilatation by inducing the formation of *S*-nitrosothiol adducts to activate guanylate cyclase (Shultz and Raij, 1989; Lahera et al., 1993) or by preventing inactivation of nitric oxide by scavenging superoxide anions (Shultz and Raij, 1989). Our findings agree with those of previous reports showing that a reduction in blood pressure is not sufficient to improve endothelial function in hypertensive humans (Panza et al., 1993) and rats (Clozel et al., 1990). However, it is possible that the increased

endothelium-dependent relaxant response to captopril may contribute to its antihypertensive action in Zucker rats.

Several studies in different models of experimental hypertension have shown beneficial effects of angiotensin-converting enzyme inhibitors (with and without sulphydryl group) in either preventing or reversing endothelial dysfunction (Lüscher and Vanhoutte, 1987; Clozel et al., 1990; Tschudi et al., 1994). However, in hypertensive subjects Creager and Roddy (1994) found no improvement of the impaired endothelial function after 2 months of therapy with captopril or enalapril. This discrepancy shows that, despite methodological differences, the mechanisms involved in the endothelial dysfunction vary depending on the hypertension model tested. The mechanism implicated in the impaired endothelial function in obese Zucker rats is not known, but might be related to an overproduction of superoxide anions. Further studies are needed to determine this.

In conclusion, we demonstrated that a two-week-treatment with the angiotensin-converting enzyme inhibitors captopril or enalapril reversed insulin resistance and the associated cardiovascular complications (cardiac hypertrophy, hypertension and proteinuria) seen in obese Zucker rat. Furthermore, at doses equieffective in reversing these complications, captopril, but not enalapril, improved the impaired endothelium-dependent and independent relaxant responses in the isolated rat aorta.

This work was supported by CICYT grants (SAF98-0157 and SAF98-0160). We are grateful to Dr. F. Pérez-Vizcaíno for the critical reading of the manuscript.

References

Anderson, E.A., Hoffman, R.P., Balon, T.W., Sinkey, C.A., Mark, A.L., 1991. Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. J. Clin. Invest. 87, 2246–2252.

Bao, G., Gohlke, P., Qadri, F., Unger, T., 1992. Chronic kinin receptor blockade attenuates the antihypertensive effect of ramipril. Hypertension 20, 74–79.

Bochicchio, T., Ron, O., Sandoval, G., Bobadilla, N., Ruiz, A., Herrera-Acosta, J., 1991. Effect of captopril on proteinuria ($U_{pro}V$) and renal hemodynamics in hypertensive type-II diabetic patients with nephropathy (DNH). Hypertension 17, 422.

Bohlen, H.G., Lash, J.M., 1995. Endothelial-dependent vasodilation is preserved in non-insulin-dependent Zucker fatty diabetic rats. Am. J. Physiol. 268, H2366–H2374.

Bray, G.A., 1977. The Zucker-fatty rat: a review. Fed. Proc. 36, 148–153.

Cachofeiro, V., Sakakibara, T., Nasjletti, A., 1992. Kinins, nitric oxide, and the hypotensive effect of captopril and ramiprilat in hypertension. Hypertension 19, 138–145.

Clozel, M., Kuhn, H., Hefti, F., 1990. Effects of angiotensin converting enzyme inhibitors and of hydralazine on endothelial function in hypertensive rats. Hypertension 16, 532–540.

Cox, R.H., Kikta, D.C., 1992. Age-related changes in thoracic aorta of obese Zucker rats. Am. J. Physiol. 262, H1548–H1556.

Creager, M.A., Roddy, M.-A., 1994. Effect of captopril and enalapril on endothelial function in hypertensive patients. Hypertension 24, 499–505.

Crettaz, M., Prentki, M., Zaninetti, D., Jeanrenaud, B., 1980. Insulin resistance in soleus muscle from obese Zucker rats: involvement of several defective sites. Biochem. J. 186, 525–534.

Duarte, J., Pérez-Vizcaíno, F., Zarzuelo, A., Jimenez, J., Tamargo, J., 1994. Inhibitory effects of quercetin and staurosporine on phasic contractions in rat vascular smooth muscle. Eur. J. Pharmacol. 262, 149–156.

Dzau, V.J., 1989. Short- and long-term determinants of cardiovascular function and therapy: contributions of circulating and tissue renin–angiotensin system. J. Cardiovasc. Pharmacol. 14, S1–S5.

Erdoes, E.G., Skidgel, R.A., 1986. The unusual substrate specificity and the distribution of human angiotensin I converting enzyme. Hypertension 8, 40–48.

Feletou, M., Moreau, N., Duhault, J., 1994. Vascular responsiveness in young, diabetic, and aging hyperinsulinemic rats. Life Sciences 54, 1801–1813.

Ferrannini, E., Buzzigoli, G., Giorico, M.A., 1987. Insulin resistance in essential hypertension. New Engl. J. Med. 31, 350–357.

Frochlich, E., Apstein, C., Chobanian, A., Devereux, R., Dustan, H., Dzau, V., Fauad-Tarazi, F., Horan, M., Marcus, M., Massie, B., Pfefer, M., Re, R., Roccella, E., Savage, D., Shub, C., 1993. The heart in hypertension. New Engl. J. Med. 327, 998–1008.

Furchgott, R.F., 1983. Role of endothelium in responses of vascular smooth muscle. Circ. Res. 53, 557–573.

Furchgott, R.F., Vanhoutte, P.M., 1989. Endothelium-derived relaxing and contracting factors. FASEB J. 3, 2007–2018.

Harker, C.T., Odonnell, M.P., Kasiske, B.l., Keane, W.F., Katz, S.A., 1993. The renin–angiotensin system in the type-II diabetic obese Zucker rat. J. Am. Soc. Nephrol. 4, 1354–1361.

Henriksen, E.J., Jacob, S., 1995. Effects of captopril on glucose transport activity in skeletal muscle of obese Zucker rats. Metabolism 44, 267–273.

Henriksen, E.J., Jacob, S., Augustin, H.J., Dietze, G.J., 1996. Glucose transport activity in insulin-resistant rat muscle. Effects of angiotensin-converting enzyme inhibitors and bradykinin antagonism. Diabetes 45, S125–S128.

Ionescu, E., Sauter, J.F., Jeanrenaud, B., 1985. Abnormal oral glucose tolerance in genetically obese (fa/fa) rats. Am. J. Physiol. 248, E500–E506.

Kaplan, N.M., 1989. The deadly quartet. Arch. Intern. Med. 149, 1514–1520.

Kasiske, B.L., Cleary, M.P., O'Donnell, M.P., Keane, W.F., 1985. Effects of genetic obesity on renal structure and function in the Zucker rat. J. Lab. Clin. Med. 106, 598–604.

Kemmer, F.W., Berger, M., Herberg, L., Gries, F.A., Wirdeier, A., Becker, K., 1979. Glucose metabolism in perfused skeletal muscle: demonstration of insulin resistance in obese Zucker rat. Biochem. J. 178, 733–741.

King, P.A., Horton, E.D., Hirshman, M.F., Horton, E.S., 1992. Insulin resistance in obese Zucker rat (fa/fa) skeletal muscle is associated with a failure of glucose transporter translocation. J. Clin. Invest. 90, 1568–1575.

Kurtz, T.W., Morris, R.C., Pershadsingh, H.A., 1989. The Zucker fatty rat as a genetic model of obesity and hypertension. Hypertension 13, 896–901.

Lahera, V., Khraiba, A.A., Romero, J.C., 1993. Sulfhydryl group donors potentiate the hypotensive effect of acetylcholine in rats. Hypertension 22, 156–160.

Levin, B.E., Stoddard-Apter, S., Sullican, A.C., 1984. Central activation and peripheral function of sympatho-adrenal and cardiovascular systems in the Zucker rat. Physiol. Behav. 32, 295–299.

Linz, W., Wiemer, G., Gohlke, P., Unger, T., Schölkens, B., 1995. Contribution of kinins to the cardiovascular actions of angiotensin-converting enzyme inhibitors. Pharmacol. Rev. 47, 25–49.

Lithell, H.O.L., 1991. Effect of antihypertensive drugs on insulin, glucose, and lipid metabolism. Diabetes Care 14, 203–209.

Lüscher, T.F., Vanhoutte, P.M., 1987. Antihypertensive therapy normal-

izes endothelium-dependent relaxations in salt-induced hypertension of the rat. Hypertension 9, 193–197, suppl. III.

Modan, M., Halkin, H., Almay, S., Lusky, A., Eshkil, M., Shitrit, A., Fuchs, A., 1985. Hyperinsulinemia: a link between hypertension, obesity and glucose intolerance. J. Clin. Invest. 75, 809–817.

O'Donnell, M.P., Kasiske, B.L., Cleary, M.P., Keane, W.F., 1985. Effects of genetic obesity on renal structure and function in the Zucker rat: II. Micropuncture studies. J. Lab. Clin. Med. 106, 605–610.

Panza, J.A., Quyyumi, A.A., Callahan, T.S., Epstein, S.E., 1993. Effect of antihypertensive treatment on endothelium-dependent vascular relaxation in patients with essential hypertension. J. Am. Coll. Cardiol. 21, 1145–1151.

Pawloski, C.M., Kanagy, N.L., Mortensen, L.H., Fink, G.D., 1992. Obese Zucker rats are normotensive on normal and increased sodium intake. Hypertension 19, I90–I95.

Peuler, J.D., Mark, A.L., 1989. Aggravation of salt-induced hypertension in Dahl rats by 2% supplemental dietary calcium. Hypertension 13, 929–934.

Reaven, G.M., 1988. Role of insulin resistance in human disease. Diabetes 37, 1595–1607.

Rosenthal, T., Erlich, Y., Rosenmann, E., Cohen, A., 1997. Effects of enalapril, losartan, and verapamil on blood pressure and glucose metabolism in the Cohen–Rosenthal diabetic hypertensive rat. Hypertension 29, 1260–1264.

Schmitz, P.G., O'Donnell, M.P., Kasiske, B.L., Katz, S.A., Keane, W.F., 1992. Renal injury in obese Zucker rats: glomerular hemodynamic alterations and effects of enalapril. Am. J. Physiol. 263, F496–F502.

Sherman, W.M., Katz, A.L., Cutler, C.L., Withers, R.T., Ivy, J.L., 1988. Glucose transport: locus of muscle insulin resistance in obese Zucker rats. Am. J. Physiol. 255, E374–E382.

Shultz, P.J., Raij, L., 1989. Effects of antihypertensive agents on endothelium-dependent and endothelium-independent relaxations. Br. J. Clin. Pharmacol. 28, 1515–1575.

Smith, R.D., Chiu, A.T., Wong, P.C., Herblin, W.F., Timmermans, P., 1992. Pharmacology of nonpeptide angiotensin II receptor antagonists. Annu. Rev. Pharmacol. Toxicol. 32, 135–165.

Tomiyama, H., Kushiro, T., Abeta, H., Ishii, T., Takahashi, A., Furukawa, L., Asagami, T., Hino, T., Saito, F., Otsuka, Y., Kurumatani, H., Kobayashi, F., Kanmatsuse, K., Kajiwara, N., 1994. Kinins contribute to the improvement of insulin sensitivity during treatment with angiotensin converting enzyme inhibitor. Hypertension 23, 450–455.

Tschudi, M.R., Criscione, L., Novosel, D., Pfeiffer, K., Lüscher, T.F., 1994. Antihypertensive therapy augments endothelium-dependent relaxations in coronary arteries of spontaneously hypertensive rats. Circulation 89, 2212–2218.

Uehara, M., Kishikawa, H., Isami, S., Kisanuki, K., Ohkubo, Y., Miyamura, N., Miyata, T., Yano, T., Shichiri, M., 1994. Effects on insulin sensitivity of angiotensin converting enzyme inhibitors with or without a sulphydryl group: bradykinin may improve insulin resistance in dogs and humans. Diabetologia 37, 300–307.

Waeber, B., Brunner, H.R., 1996. Cardiovascular hypertrophy: role of angiotensin II and bradykinin. J. Cardiovasc. Pharmacol. 27, S36–S40.

Vallance-Owen, J., 1972. Estimation of plasma-insulin by the rat diaphragm method. Lancet 9, 68–70.

Walker, A.B., Dores, J., Buckingham, R.E., Savage, M.W., Williams, G., 1997. Impaired insulin induced attenuation of noradrenaline-mediated vasoconstriction in insulin-resistant obese Zucker rats. Clin. Sci. 93, 235–241.

Wu, X., Makynen, H., Kahonen, M., Arvola, P., Porsti, I., 1996. Mesenteric arterial function in vitro in three models of experimental hypertension. J. Hypertens. 14, 365–372.

Zarzuelo, A., Jiménez, I., Gámez, M.J., Utrilla, P., Fernandez, I., Torres, M.I., Osuna, I., 1996. Effects of luteolin 5-*O*-β-rutinoside in streptozotocin-induced diabetic rats. Life Sciences 58, 2311–2316.

Zemel, M.B., Reddy, S., Shehin, S.E., Lockette, W., Sowers, J.R., 1990. Vascular reactivity in Zucker rats: role of insulin resistance. J. Vasc. Med. Biol. 2, 81–85.

Zemel, M.B., Reddy, S., Sowers, J.R., 1991. Insulin attenuation of vasoconstrictor responses to phenylephrine in Zucker lean and obese rats. Am. J. Hypertens. 4, 537–539.

Zemel, M.B., Peuler, J.D., Sowers, J.R., Simpson, L., 1992. Hypertension in insulin-resistant Zucker obese rats is independent of sympathetic neural support. Am. J. Physiol. 262, E368–E371.