ELSEVIER

European Journal of Pharmacology 420 (2001) 133–141

ejp

www.elsevier.nl/locate/ejphar

Chronic administration of losartan plus hydrochlorothiazide improves vascular status in young cardiomyopathic hamsters

María J. Crespo *, Walmor C. De Mello

Department of Pharmacology, University of Puerto Rico-School of Medicine, GPO Box 365067, San Juan 00936-5067, Puerto Rico

Received 6 February 2001; received in revised form 12 April 2001; accepted 18 April 2001

Abstract

The combination of an angiotensin II receptor antagonist and a thiazide has been used extensively in the treatment of patients with overt heart failure. The effect of this combination on the vascular wall early in the disease, however, has not been investigated. To evaluate this effect, the vascular status of 3-month-old cardiomyopathic hamsters was assessed after daily administration of a combination of losartan (25 mg/kg, p.o.) and hydrochlorothiazide (6.5 mg/kg, p.o.) over an 8-week period. Age-matched golden hamsters were used as healthy controls. The contractile response of aortic rings to endothelin-1 was significantly higher in cardiomyopathic hamsters than in control animals. Concentration–response curves for the endothelin-1-induced contraction were displaced to the right after hydrochlorothiazide + losartan treatment (toward the curves for healthy controls); however, E_{max} from treated hamsters was significantly reduced when compared to E_{max} from untreated cardiomyopathic animals (1.016 ± 0.073 vs. 1.346 ± 0.153 g, $P < 0.05$, $n = 6$). No significant differences in the EC_{50} values from these curves were observed between hydrochlorothiazide + losartan treated and untreated cardiomyopathic animals (2.90 ± 0.95 vs. 1.10 ± 0.85 nM, $P > 0.05$). The acetylcholine-induced relaxation observed in cardiomyopathic animals was not improved after treatment with hydrochlorothiazide + losartan or hydrochlorothiazide alone, but the combination of these drugs increased significantly the basal production of nitric oxide (NO). Angiotensin-converting enzyme activity increased in plasma (from 29.9 ± 1.23 to 41.16 ± 1.82 nmol mg^{-1} min^{-1}, $n = 8$, $P < 0.05$) but decreased in the aorta (from 0.33 ± 0.02 to 0.25 ± 0.017 nmol mg^{-1} min^{-1}, $n = 6$, $P < 0.05$) after treatment with hydrochlorothiazide + losartan. In addition, the combination of these drugs reduced the heart-to-body mass ratio (3.96 ± 0.07 for treated vs. 5.01 ± 0.20 mg/g for untreated animals, $n = 7$, $P < 0.05$), and the thickness of the aortic media (0.076 ± 0.003 for treated vs. 0.149 ± 0.009 mm for untreated animals, $n = 8$, $P < 0.05$). Although hydrochlorothiazide alone lowered systolic blood pressure to the same level achieved with both drugs in combination (from 166 ± 10 for untreated cardiomyopathic animals to 84 ± 1 mm Hg for hydrochlorothiazide + losartan, and 80 ± 5 mm Hg for hydrochlorothiazide alone, $P < 0.05$), no significant reduction in heart-to-body mass ratio was observed in animals treated with the diuretic alone ($P > 0.05$). In conclusion, in this model of heart failure, chronic hydrochlorothiazide + losartan administration normalizes the vascular responses to endothelin-1, improves basal vascular tone, and prevents the development of cardiac and vascular hypertrophy. © 2001 Published by Elsevier Science B.V.

Keywords: Vascular function; Losartan; Hydrochlorothiazide; Heart failure; Cardiomyopathic hamster

1. Introduction

Heart failure is a cardiovascular condition that, despite massive efforts by the health community, is increasing within the general population (McMurray et al., 1993). Different pharmacological approaches have been implemented during the last decades to decrease morbidity and mortality in these patients. The use of non-peptide angiotensin II Type 1 receptor (AT_1) antagonists has proven to be beneficial in the treatment of patients with heart failure. The Evaluation of Losartan in the Elderly-II trial (ELITE-II) demonstrated that in older patients with heart failure, the mortality in the group of patients treated with the angiotensin AT_1 receptor antagonist losartan was not significantly reduced when compared with the mortality in the captopril (an angiotensin-converting enzyme inhibitor) group (Pitt et al., 2000). The compliance of the losartan group was significantly greater, however, due to a significant reduction on secondary effects. A significant number of heart failure patients are now being treated daily with an angiotensin AT_1 receptor antagonist and a diuretic, in

* Corresponding author. Tel.: +1-787-766-4441; fax: +1-787-282-0568.

E-mail address: mcrespo@rcm.upr.edu (M.J. Crespo).

0014-2999/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0014-2999(01)01006-8

particular, hydrochlorothiazide (Oparil et al., 1996). Hydrochlorothiazide is known, however, to raise plasma renin activity (Kohzuki et al., 1996; Traub et al., 1976). This action may, in turn, activate the renin–angiotensin system and oppose the effects of the angiotensin AT_1 receptor antagonists. In addition, hydrochlorothiazide-induced activation of the renin–angiotensin system may sensitize patients to inhibitors of this system, including the angiotensin AT_1 receptor antagonists. The majority of the published studies focus on the effect of the combination of hydrochlorothiazide and losartan on the hemodynamic profile of patients and animal models with patent heart failure. The impact of this pharmacological intervention on the status of the vascular wall is not well documented and the effect of the administration of these drugs from the onset of the condition has not been established.

The present study was designed to assess the effect of daily administration over an 8-week period of a combination of hydrochlorothiazide + losartan on 4-week-old cardiomyopathic hamsters. Vascular reactivity to endothelin-1, vascular basal tone, blood pressure and histologic profiles were investigated in these animals. Heart-to-body mass ratio was also evaluated as a marker for cardiac hypertrophy. This study indicates that the administration of hydrochlorothiazide + losartan at the earliest stages of heart failure is beneficial because these drugs, in combination, reduced vascular and cardiac remodeling and improved vascular function in cardiomyopathic hamsters.

2. Materials and methods

2.1. Experimental animals

Male Syrian cardiomyopathic hamsters (BIO, TO-2 strain) from Bio Breeders (Fitchdurg, MA, USA) were used as a genetic model of heart failure. The cardiomyopathic Syrian hamster model was chosen because it has a genetically transmitted form of cardiomyopathy, and it is particularly useful for studies addressing the earliest stages of heart failure (Crespo et al., 1997; Crespo, 1999; De Mello and Crespo, 1999). This model presents a well-documented (Gertz, 1972; Forman et al., 1972; Strobeck et al., 1979) cardiomyopathy that follows a stereotypic sequence of events: (1) immaturity (<30 days after birth), (2) acute focal myolysis of the myocardium (30–60 days), (3) fibrosis and calcifications of necrotic patches (60–90 days), (4) ventricular hypertrophy (90–150 days), and (5) congestive heart failure (>150 days). Heart failure in these animals is not documented at the age chosen (4 weeks) to start the treatment. Cardiomyopathic hamsters were divided into three groups. The first group consisted of 12-week-old animals (myolitic phase, prehypertrophic stage, $n=14$) that were treated with hydrochlorothiazide + losartan in an 8-week period. Drugs were administered daily by gavage at a dose of 25 mg/kg (losartan) and 6.25 mg/kg (hydrochlorothiazide). The composition of the second group was the same as the first group in age and number, but these animals received only the diuretic (hydrochlorothiazide, 6.25 mg/kg, p.o.). A third group of animals was treated only with the vehicle and was used as untreated cardiomyopathic controls. In addition, age-matched BIO, F1-B golden hamsters (Bio Breeders) were used as healthy controls. All animals were housed in a temperature-controlled room, on a 12-h light/dark cycle. Commercial rat chow and tap water were available ad libitum. At the time of the experiments, all animals were 12–14 weeks old. The investigation conforms to the Guide and Care for the Use of Laboratory Animals published by US National Institutes of Health.

2.2. Experimental procedures

On the same day of the experiment, animals were weighed and anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). When complete anesthesia was achieved, the thorax was opened, and the heart and descending thoracic aorta were removed. The heart was then weighed. Isolated aortas were placed in a Krebs' bicarbonate solution (composition in mM: 118 NaCl, 2.5 $CaCl_2$, 5 KCl, 1.1 $MgSO_4$, 25 $NaHCO_3$, 1.2 KH_2PO_4 and 10 glucose, pH = 7.4). The connective tissue adjacent to the aortic adventitia was carefully removed, avoiding damage to the smooth muscle and the endothelium. An aortic ring, approximately 5 mm in length, was obtained from the proximal segment of each aorta and used for the contractility studies.

2.3. Measurement of aortic ring relaxation and contraction

Aortic rings were suspended horizontally between two stainless-steel wires. One wire had a static position, whereas the other wire was connected to a force-displacement transducer (Grass, model FT03C) and attached to a Grass DC preamplifier model 7P1F. The signal was analyzed by a National Instruments PC-LPM-16/PnP data acquisition card, and the changes in isometric tension were recorded using the program LabView. The whole preparation was mounted in a two-hook 50-ml organ chamber (Radnoti, Monrovia, CA) and bathed in Krebs' bicarbonate solution (aerated with a mixture of 95% O_2 and 5% CO_2 at 37°C). The rings were subjected to a tension of 1.0 g at rest. This tension was assessed previously in our laboratory as the optimal tension for these experiments. Once the optimal tension was reached, the aortic rings were subjected to a 1-h equilibration period. The effect of the treatment on the endothelin-1-induced contraction was tested by analyzing the cumulative concentration–response curves generated by the peptide (0.1 nM to 1.0 μM) in aortic rings from treated and untreated cardiomyopathic animals, and from healthy controls. Sodium nitroprusside (1.0 μM) was used to fully relax aortic rings after comple-

tion of the above protocol. The concentrations inducing 50% of maximal contraction (EC_{50}) for endothelin-1 were determined through analysis of the concentration–response curves for each individual group. To assess the status of the endothelium-dependent relaxation, cumulative concentration–response curves (0.1 nM to 10.0 μM) for acetylcholine were generated in aortic rings precontracted with 0.1 μM norepinephrine. This procedure was performed for each experimental group. To determine the effect of hydrochlorothiazide + losartan on basal tone, the rings were subjected to a resting tension of 1.0 g, equilibrated for 1 h, and incubated with 1 mM N^G-nitro-L-arginine (L-NAME) for a 45-min period. L-NAME is an arginine analog that inhibits nitric oxide (NO) synthase. The initial resting tension was compared with the tension after the 45-min incubation with L-NAME for each experimental group. Analysis of these results allows the determination of the status of the vascular basal production of NO.

2.4. Measurement of plasma and tissue angiotensin-converting enzyme activity

Angiotensin-converting enzyme activity was determined using the fluorometric assay described previously by Cushman and Cheung (1991). In this method, angiotensin-converting enzyme activity is correlated with the rate of generation of the dipeptide His–Leu from Hip–His–Leu substrate by the enzyme. Angiotensin-converting enzyme-activity was determined in plasma and aorta homogenates from treated and untreated cardiomyopathic hamsters, as well as in age-matched golden controls. Vascular homogenates were prepared by adding 1 g of tissue to 10 ml of ice-cold 50 mM potassium phosphate buffer (50 mM, pH = 7.5). An aliquot (100 μl) of the homogenate was added to 100 μl of Hip–His–Leu (12.5 mM), and a final volume of 250 μl was achieved by the addition of 50 μl of water. The mixture was incubated for 10 min at 37°C. The reaction was then stopped with 1.45 ml of NaOH (280 mM). Following this stage, 1% phthalaldehyde was added, and the mixture was incubated for 10 min at room temperature. Subsequently, 200 μl of HCl (3N) was added to the mixture. After a final 30-min incubation period, the medium was excited at 364 nm, and the fluorescence was determined at 486 nm using a Shimadzu RF5000U spectrofluorometer. Aortic angiotensin-converting enzyme activity was determined in minced tissue from the distal part of the abdominal aorta. Plasma angiotensin-converting enzyme activity values were determined by the addition of 10 volumes of phosphate buffer to 1 ml plasma samples. To quantify angiotensin-converting enzyme-activity, a calibration curve was performed using variable His–Leu concentrations from 0 to 3 nM.

2.5. Noninvasive determination of systolic blood pressure

For each experimental group of hamsters, systolic blood pressure was determined in the femoral artery by placing a pressure cuff on the left leg. The cuff was inflated until a pressure of 250 mm Hg was achieved. Systolic blood pressure was then recorded by a piezoelectric sensor placed near the pressure cuff. The sensor was connected to a microcomputer system (RTBP-2000, Kent Scientific, Litchfield, CT) that processed the signals. The data were recorded and analyzed using the program LabView. This setup allows consecutive determinations of systolic blood pressure in the same animal. An average value of five determinations separated by a 3-min interval was reported for each animal.

2.6. Determination of nitric oxide basal production

The basal endothelial NO production was correlated with the plasmatic nitrate/nitrite concentration using the method described previously by Tracey et al. (1995). Nitrate/nitrite concentrations were quantified using the Griess reagent (1% sulphanilamide in 5% H_3O_4 and 0.1% naphthylnediamine dihydrochloride, in a ratio of 1:1). Animal blood was centrifuged at 3000 rpm for 5 min and plasma samples were stored in the refrigerator overnight at 4°C. A 750-μl aliquot of plasma was mixed with the same volume of Griess reagent and incubated for 15 min at room temperature in the absence of light. The samples were then quantified spectrophotometrically at 540 nm.

2.7. Histological techniques

Rings and longitudinal sections from the aorta of treated and untreated cardiomyopathic hamsters and from age-matched healthy controls were fixed in formalin and embedded in paraffin. Five-micrometer sections were stained with Masson trichrome (specific for collagen fibers) and von Kossa's Ca^{2+} stain, and then photographed. For each sample, the thickness of the aortic media was measured in several locations using a computer software (SigmaScan Pro), and then averaged.

2.8. Drugs

The following drugs were obtained from Sigma (St. Louis, MO): endothelin-1, N^G-nitro-L-arginine (L-NAME), potassium chloride, norepinephrine, sodium chloride, hydrochlorothiazide, calcium chloride, acetylcholine, magnesium sulfate, sodium bicarbonate, potassium monobasic phosphate, glucose, and sodium nitroprusside. Losartan and hydrochlorothiazide (in the form of Hyzaar) were donated by Merck, Sharp and Dohme.

2.9. Statistical analysis

Results are presented as the mean ± S.E.M. EC_{50} values were determined by graphical analysis (GraphPAD, CA, USA). Statistical comparisons between groups were performed with Student's *t*-test when only two variables were compared and with the analysis of variance when

more than two variables were compared. Values were considered statistically significant at $P < 0.05$.

3. Results

3.1. Effect of losartan + hydrochlorothiazide on endothelin-1-induced contraction

Cumulative concentration–response curves for endothelin-1 were performed in order to determine the effect of hydrochlorothiazide + losartan on the vascular response to this peptide (Table 1). As depicted in Fig. 1, the endothelin-1 curve from untreated cardiomyopathic animals was displaced to the left compared to that obtained from healthy controls (EC_{50}: 1.10 ± 0.85 and 6.04 ± 1.6 nM, respectively, $P < 0.05$). In addition, the contraction recorded for each individual endothelin-1 concentration was enhanced in cardiomyopathic animals compared to healthy controls ($P < 0.05$). When treated with hydrochlorothiazide + losartan, however, the vascular response to endothelin-1 decreased in cardiomyopathic hamsters. The concentration–response curve from treated animals was significantly displaced to the right, toward the healthy controls curve, compared to the curve from untreated cardiomyopathic hamsters. Nevertheless, the EC_{50} values remain unchanged (2.90 ± 0.95 vs. 1.10 ± 0.85 nM, for treated and untreated cardiomyopathic hamsters, respectively, $P > 0.05$). These results indicate that, whereas the combination of these drugs does not modify the affinity of endothelin-1 for its receptors, the contractile response to this peptide is decreased, approaching those responses found in healthy controls.

3.2. Effect of losartan + hydrochlorothiazide on endothelial-dependent relaxation and basal NO production

In order to evaluate the effect of hydrochlorothiazide + losartan on endothelial function, the relaxing effect of acetylcholine was assessed in 0.1 μM norepinephrine-precontracted aortic rings from age-matched treated and untreated cardiomyopathic hamsters, as well as in healthy controls. The effect of acetylcholine was expressed as a percentage of the relaxation relative to the maximal contraction induced by 0.1 μM norepinephrine. The concentration response curves for untreated cardiomyopathic animals were significantly displaced to the right compared with the curves from healthy controls (Fig. 2, Table 2). No significant differences were found in the EC_{50} values from either curve (20.0 ± 6.6 for healthy controls vs. 38.3 ± 6.0 nM for untreated cardiomyopathic animals, $n = 7$, $P >$

Table 1
Effect of losartan and hydrochlorothiazide on the EC_{50} and E_{max} for the endothelin-1-induced vascular contraction

Experimental group	EC_{50} (nM)	E_{max} (g)
Control hamsters		
No treatment	6.04 ± 0.8	0.83 ± 0.05
Cardiomyopathic hamsters		
No treatment	1.10 ± 0.85	1.34 ± 0.15
Hydrochlorothiazide + losartan	2.90 ± 0.9	1.01 ± 0.07[a]

EC_{50}: concentration of the drug necessary to obtain 50% of the maximal response.
E_{max}: maximal contraction.
[a] $P < 0.05$ for comparison of hydrochlorothiazide + losartan treated hamsters with untreated cardiomyopathic animals.

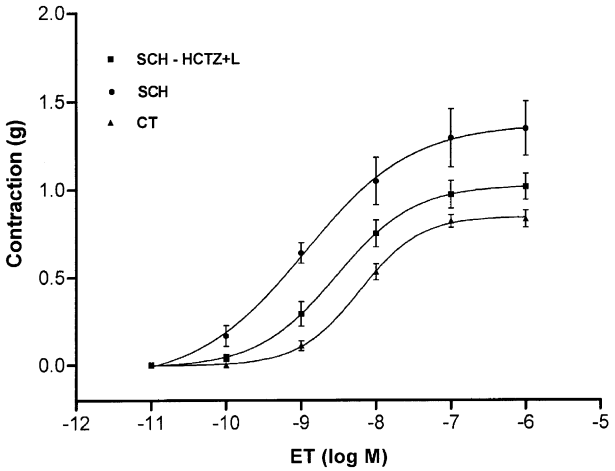

Fig. 1. Cumulative concentration response curves for endothelin-1 (ET-1)-induced contraction in aortic rings from untreated cardiomyopathic hamsters (SCH), from SCH treated with hydrochlorothiazide + losartan (HCTZ + L), and from age-matched healthy controls (CT). The values shown are the means ± S.E.M. of six experiments. Note that, in treated cardiomyopathic animals, the response to ET-1 decreases for each individual concentration ($P < 0.05$), and the curve shifts to the right towards the curve from healthy controls.

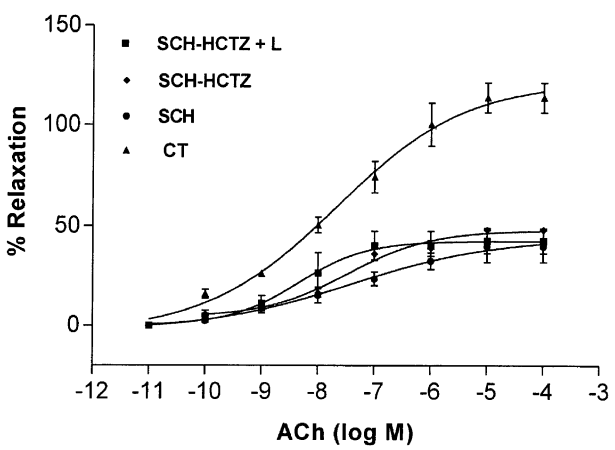

Fig. 2. Cumulative concentration response curves for acetylcholine (ACh)-induced relaxation in untreated cardiomyopathic hamsters (SCH), in SCH treated with hydrochlorothiazide + losartan (HCTZ + L), in SCH treated HCTZ alone, and in age-matched healthy controls (CT). The values shown are the means ± S.E.M. of seven experiments. Aortic rings were precontracted with 0.1 μM norepinephrine before the addition of cumulative concentrations of ACh.

Table 2
Effect of losartan and hydrochlorothiazide on the EC_{50} and percentage of maximal relaxation induced by acetylcholine

Experimental group	EC_{50} (nM)	Percentage maximal relaxation
Control hamsters		
No treatment	20.8 ± 6.6	113.8 ± 7.54
Cardiomyopathic hamsters		
No treatment	38.33 ± 6.0	40.01 ± 8.0[a]
Hydrochlorothiazide + losartan	4.51 ± 0.8[a]	42.64 ± 6.19[a]
Hydrochlorothiazide	33.96 ± 0.26	48.00 ± 1.47[a]

EC_{50}: concentration of the drug necessary to obtain 50% of the maximal relaxation.
[a] $P < 0.05$ for comparison between these groups and the control group.

0.05). Cardiomyopathic hamsters achieved only a 40.01 ± 8.0% ($n = 7$) maximal relaxation with 10 μM acetylcholine concentration, however, compared to 113.8 ± 7.5% ($n = 7$, $P < 0.05$) obtained for healthy controls. After hydrochlorothiazide + losartan treatment, cardiomyopathic hamsters did not show a significant improvement in endothelial function, although the EC_{50} value from the acetylcholine-induced relaxation curve was significantly decreased (4.51 ± 0.8 nM, $P < 0.05$). Similarly, hydrochlorothiazide alone did not improve endothelial-dependent relaxation and the curve was superimposable on those from hydrochlorothiazide + losartan and untreated cardiomyopathic animals. Whereas hydrochlorothiazide + losartan treatment produced no significant improvement in acetylcholine-induced relaxation, its effect on basal tone was dramatically different. Fig. 3 shows the result of the 45-min incubation with L-NAME on basal tension of the aorta. Inhibition of the NO synthase by L-NAME in aortic rings from treated animals produced a 0.22 ± 0.03 g ($n = 8$, $P < 0.05$) increment in basal resting tension. In contrast, no effect was observed on resting tension in untreated cardiomyopathic animals when NO synthase was inhibited. Comparable results were obtained when the concentration of nitrates/nitrites, as an indirect measure of basal NO production, was assessed in the plasma of treated (hydrochlorothiazide + losartan and hydrochlorothiazide alone) and untreated animals. Table 3 shows that NO concentrations in hydrochlorothiazide treated and untreated cardiomyopathic animals were similar (6.69 ± 0.31 and 6.07 ± 0.98 μM, respectively). However, these values increased significantly after hydrochlorothiazide + losartan treatment (9.05 ± 077 μM), but did not reach control values (17.77 ± 4.2 μM).

3.3. Effect of losartan + hydrochlorothiazide on plasmatic and aortic angiotensin-converting enzyme activity

The status of both local and circulating renin–angiotensin system was evaluated in cardiomyopathic hamsters before and after treatment with hydrochlorothiazide + losartan. As illustrated in Fig. 4A and B, the pattern of angiotensin-converting enzyme activity in the aorta differed from that of plasma after treatment. Angiotensin-converting enzyme activity decreased in aortic tissue from 0.33 ± 0.02 in untreated hamsters to 0.25 ± 0.017 nmol mg^{-1} min^{-1} in treated animals ($P < 0.05$), but increased in plasma from 29.9 ± 1.2 in untreated animals to 41.2 ± 1.8 nmol mg^{-1} min^{-1} in treated animals ($P < 0.05$). Values for control hamsters (CT) were 0.27 ± 0.02 and 21.15 ± 0.52 nmol mg^{-1} min^{-1} for the aorta and plasma, respectively.

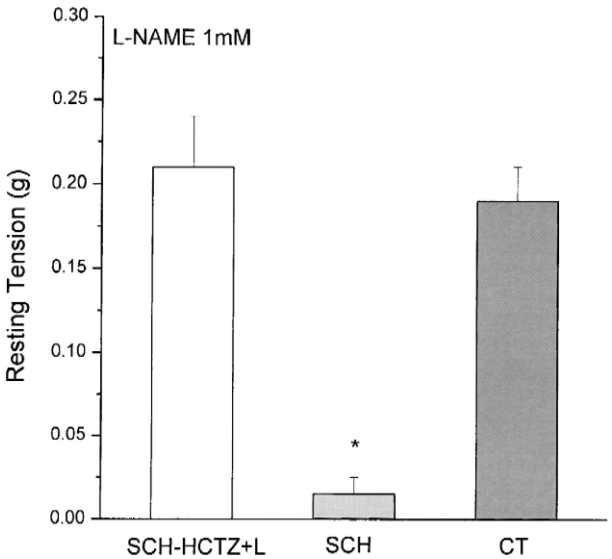

Fig. 3. Effect of 1 mM L-NAME on resting tension developed in aortic rings from untreated cardiomyopathic hamsters (SCH), from SCH treated with hydrochlorothiazide + losartan (HCTZ + L), and from healthy controls (CT). Rings were equilibrated at 1 g basal tension and incubated with 1 mM N^G-nitro-L-arginine (L-NAME) for 45 min. Note that basal resting tension significantly increases in rings from both treated cardiomyopathic hamsters and controls after the incubation period.

Table 3
Heart-to-body mass ratio, blood pressure, and plasmatic nitric oxide (NO) concentration of experimental groups
The values are the means ± S.E.M. of seven experiments.

Experimental group	Heart-to-body mass ratio (mg/g)	Blood pressure (mm Hg)	Plasma [NO] (μM)
Control hamsters			
No treatment	3.57 ± 0.16	110 ± 7	17.77 ± 4.2
Cardiomyopathic hamsters			
No treatment	5.01 ± 0.20[a]	166 ± 10[b]	6.07 ± 0.98
Hydrochlorothiazide + losartan	3.96 ± 0.07	84 ± 1	9.05 ± 0.77
Hydrochlorothiazide	4.77 ± 0.25[c]	80 ± 5	6.69 ± 0.31

[a] $P < 0.05$ for comparison of untreated cardiomyopathic hamsters with the control group and cardiomyopathic animals treated with hydrochlorothiazide + losartan.
[b] $P < 0.05$ for comparison of untreated cardiomyopathic hamsters with all other groups.
[c] $P > 0.05$ for comparison of hydrochlorothiazide treated hamsters with untreated cardiomyopathic animals.

3.4. Effect of losartan + hydrochlorothiazide on aortic wall thickness and heart-to-body mass ratio

Histological studies performed on 5-μm sections of the upper part of the thoracic aorta of 3-month-old cardiomyopathic and control hamsters revealed that the thickness of the media was significantly reduced after hydrochlorothiazide + losartan treatment. The thickness of the media was 0.076 ± 0.003 in treated cardiomyopathic vs. 0.149 ± 0.009 mm in untreated cardiomyopathic hamsters ($n = 8$, $P < 0.05$). As depicted in Fig. 5, a significant regression of the hypertrophy present in the aorta was observed. The thickness of the media after hydrochlorothiazide + losartan treatment was similar to values obtained for age-matched controls (0.098 ± 0.004 mm). Interstitial fibrosis and calcification were not observed in the wall of the aorta of treated or untreated animals at this age. Results from heart-to-body mass ratio exhibited the same pattern (see Table 3). The combination of hydrochlorothiazide and losartan significantly reduced this ratio when compared to values from untreated cardiomyopathic hamsters (from 5.01 ± 0.20 for untreated to 3.96 ± 0.07 mg/g for treated, $P < 0.05$). In contrast, hydrochlorothiazide alone failed to reduce cardiac hypertrophy because the values for heart-to-body mass ratio from untreated and hydrochlorothiazide treated cardiomyopathic animals were not statistically significant (5.01 ± 0.20 and 4.77 ± 0.25 mg/g, respectively, $P > 0.05$).

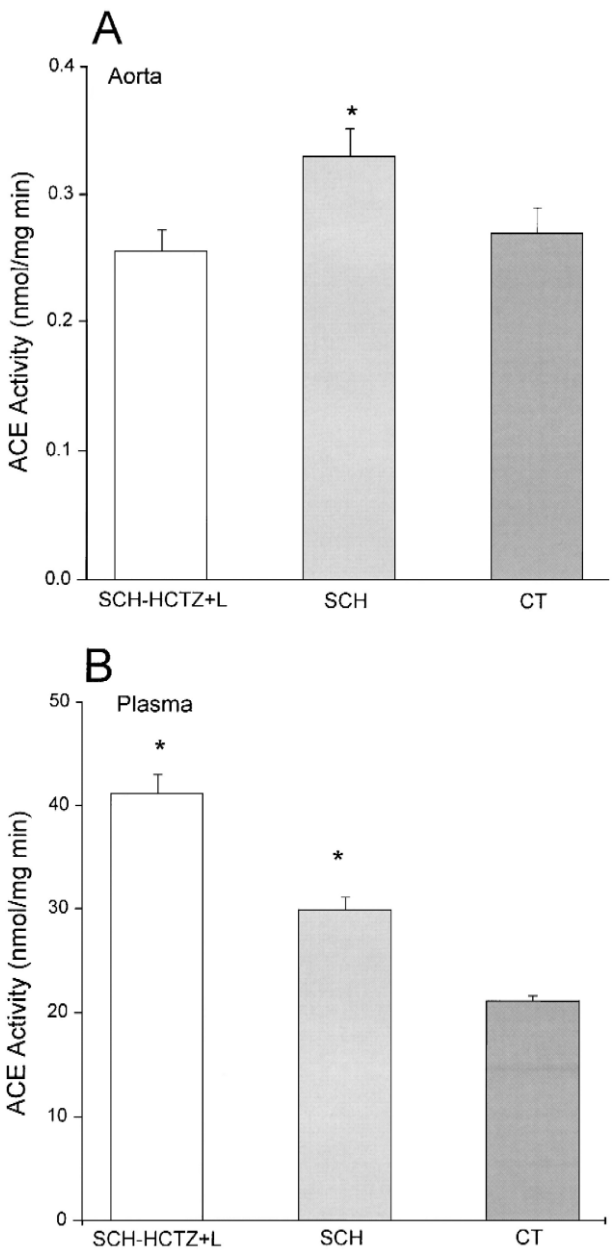

Fig. 4. (A) Angiotensin-converting enzyme activity (nmol mg^{-1} min^{-1}) in aorta homogenates from untreated cardiomyopathic animals (SCH), from SCH treated with hydrochlorothiazide + losartan (HCTZ + L), and from healthy controls (CT). ACE activity significantly decreases after treatment (0.25 ± 0.017 vs. 0.33 ± 0.02 nmol mg^{-1} min^{-1}, $n = 6$, $P < 0.05$). (B) Angiotensin-converting enzyme (ACE) activity (nmol mg^{-1} min^{-1}) in plasma homogenates from untreated cardiomyopathic animals (SCH), from SCH treated with hydrochlorothiazide + losartan (HCTZ + L), and from healthy controls (CT). HCTZ + L treatment significantly increases ACE activity in the plasma of cardiomyopathic animals (41.16 ± 1.82 vs. 29.9 ± 1.23 nmol mg^{-1} min^{-1}, $n = 8$, $P < 0.05$).

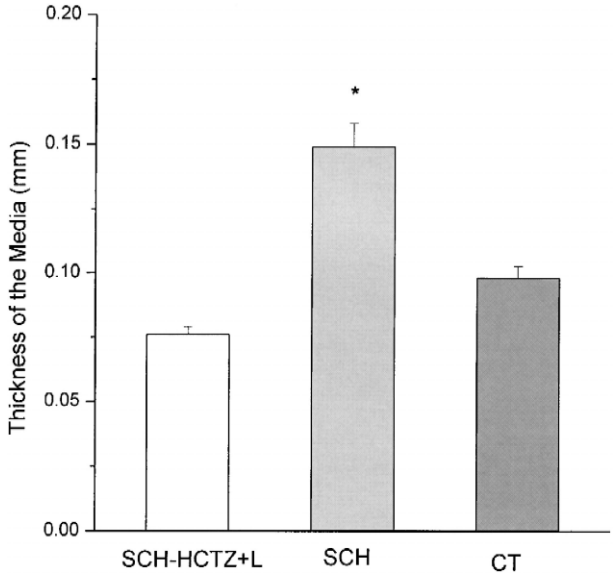

Fig. 5. Thickness of the aortic media (mm) from untreated cardiomyopathic animals (SCH), from SCH treated with hydrochlorothiazide + losartan (HCTZ + L), and from healthy controls (CT). Note that media thickness in the aorta of cardiomyopathic animals is significantly reduced after treatment, and the values approach those from control animals.

4. Discussion

Decrease in the heart-to-body ratio, as well as in the thickness of the aortic media, indicates that cardiac and vascular hypertrophy are reduced by the combination of hydrochlorothiazide + losartan. The regression of cardiac and vascular hypertrophy is likely to be attributed mainly to losartan because cardiac hypertrophy was not reduced in

the hydrochlorothiazide treated group. Regression or reduction of cardiac and vascular hypertrophy has been observed previously in different animal models after blockade of the renin–angiotensin system. In spontaneously hypertensive rats, treatment with enalapril or losartan results in near normalization of the media-to-lumen ratio in the mesenteric artery (Rizzoni et al., 1998) and reduction in cardiac hypertrophy (Schieffer et al., 1994). Losartan may reduce cardiac and vascular hypertrophy by a dual mechanism: directly, by blocking the hypertrophic actions of angiotensin II in cardiac myocytes (Liu et al., 1998) and smooth muscle, and indirectly, by diminishing the hyperreactivity of the vessel to angiotensin II and endothelin-1 (Oriji, 1999). Moreover, the improvement in heart-to-body ratio it is not related to the effect of hydrochlorothiazide. This conclusion supports previous findings with hydrochlorothiazide in cardiomyopathic hamsters (Hanton et al., 1993) and in stroke-prone, spontaneously hypertensive rats (Anderson et al., 1999). Moreover, Kohzuki et al. (1996) also found that hydrochlorothiazide therapy does not prevent or reverse cardiac hypertrophy in rats with chronic myocardial infarction. The increase in angiotensin-converting enzyme activity found in plasma from hydrochlorothiazide + losartan treated hamsters however, may be attributed to the administration of either losartan (Opsahl et al., 1995) or hydrochlorothiazide (Martinez-Maldonado et al., 1990).

The improvement in basal tension observed after treatment with hydrochlorothiazide + losartan is secondary to an increase in basal endothelial production of NO because L-NAME increases the resting tension in the vessels of these animals. This finding was confirmed in separate experiments assessing plasmatic NO concentrations. This report is the first to demonstrate that basal production of NO is restored after the administration of this combination of drugs. This beneficial effect could be mediated primarily by losartan because it is not observed in cardiomyopathic hamsters treated with hydrochlorothiazide alone. The improvement on basal resting tension seems to be unrelated to levels of systolic blood pressure since similar decreases in blood pressure were found after treatment with hydrochlorothiazide + losartan and with hydrochlorothiazide alone. Thiazides lower peripheral resistance through activation of the Ca^{2+}-dependent K^+ channels that are endothelium-independent (Calder et al., 1992). Losartan, however, has been proven to increase endothelial NO production in several different vascular beds (Rodrigo et al. 1997; Richer et al., 1996). Angiotensin AT_1 receptor antagonism has been suggested to stimulate a vasodilator cascade of bradykinin, NO and cyclic GMP through secondary activation of angiotensin AT_2 receptors (Carey et al., 2000a,b; Israel et al., 2000). Indeed, Carey et al. (2000a) state that in angiotensin II-dependent hypertension, tonic endogenous stimulation of the angiotensin AT_2 receptor protects the vasculature from further increases in blood pressure. They also suggest that one of the hypotensive actions of angiotensin AT_1 receptor blockade is mediated via the angiotensin AT_2 receptor, and that NO is the main candidate for the counter-regulatory actions of angiotensin II (via AT_2 receptor) in the vasculature. The results of the present study agree with these suggestions. Furthermore, the finding that hydrochlorothiazide + losartan treatment does not modify the acetylcholine-induced NO synthesis may also indicate that the main mechanism for endothelial improvement is related to increased NO production secondary to angiotensin AT_2 receptor activation, and not to the activation of muscarinic receptors. Additional experiments adding an angiotensin AT_2 receptor antagonist, however, are needed to confirm this notion.

Controversy still exists regarding the actions of endothelin-1 on the vasculature. Whereas Noll et al. (1994) reported abnormal reactivity of the aorta and mesenteric arteries to endothelin-1 in cardiomyopathic hamsters with pulmonary congestion, Fontaine et al. (1998), found that this peptide does not play a significant role in the coronary abnormalities observed in the UM-X7.1 cardiomyopathic hamster model. On the contrary, the latter investigators state that this peptide appears to contribute to the maintenance of myocardial contractility. The present study shows that endothelin-1 enhances vascular tone in young cardiomyopathic animals. This finding is relevant because the abnormal vascular tone precedes the appearance of clinical signs and symptoms of heart failure in this animal model and may be linked to the etiology of this condition. The discrepancies found in the literature regarding the actions of endothelin-1 on the vasculature may be attributed to the use of different experimental models, differences in regulation between the coronary arteries and other vascular beds (Tsutsui et al., 1990), and heterogeneity of the receptor population in the different tissues under study (Lin et al., 1991).

Chronic administration of losartan plus hydrochlorothiazide has been found to shift the endothelin-1 concentration–response curves toward the curves for healthy animals, without modifying the EC_{50} values. This effect appears to be mediated primarily by losartan, because hydrochlorothiazide does not alter endothelin-1 levels (Benigni et al., 1999). However, when aortic rings from spontaneously hypertensive rats are incubated with losartan, the enhanced contractile response to endothelin-1 is significantly reduced (Maeso et al., 1997). In addition, the tissue content of endothelin-1 was found to be increased in aortas from angiotensin II-induced hypertensive rats, and normalized after losartan treatment (D'Uscio et al., 1998). It is also possible that the reduced contraction observed with endothelin-1 after treatment with hydrochlorothiazide + losartan could be due to the effect of losartan blocking thromboxane A_2 receptors (Tripodi et al., 1999). It has been reported that part of the contractile effects of endothelin-1 on the vascular tissue can be attributed to the facilitation of thromboxane A_2 synthesis or release (Schmect et al., 1999). The possibility that losartan facili-

tates the release of vasodilator prostaglandins also needs to be considered. In the presence of losartan, angiotensin II interacts with the D-[Ala^7]-Ang-(1–7) (DALA)-sensitive site to promote eicosanoid release (Iyer et al., 2000). Thiazides, however, have an opposite effect because they lowered prostaglandin I_2 synthase activity in the vasculature of spontaneously hypertensive rats (Numabe et al., 1989). The interaction between angiotensin II and end endothelin-1 appears to be common to different cardiovascular diseases (e.g. hypertension, heart failure), and not a variable associated with the genetic characteristics of cardiomyopathic animals. The precise mechanisms that regulate this interaction are still unknown. Recent experiments by Ferri et al. (1999) suggest that angiotensin II regulates endothelin-1 release by cultured endothelial cells through an angiotensin AT_1 receptor-dependent pathway, while the elucidation of a dual endothelin-1/angiotensin II receptor (Ruiz-Opazo et al., 1998) supports a molecular link between these hormonal systems. In addition, a positive-feedback loop linking endothelin-1 and angiotensin II has been suggested to be present in pathological conditions, such as heart failure (Haynes and Webb, 1998). Further experiments are needed to reveal the precise mechanisms responsible for the relationship between these two peptides to define their interactions in normal and pathological states.

The results of the current study demonstrate that chronic administration of losartan combined with hydrochlorothiazide improves vascular function in 3-month-old cardiomyopathic hamsters. Specifically, these drugs normalize the increased contractile response to endothelin-1, decrease basal tone in the aorta, and prevent vascular and cardiac hypertrophy. Therefore, the combination of these drugs is likely to improve the cardiovascular status of patients from the first stages of heart failure.

Acknowledgements

This work was supported by Grants from the National Institutes of Health (RR-03051) and Merck, Sharp and Dohme.

References

Anderson, N.H., Devlin, A.M., Graham, D., Morton, J.J., Hamilton, C.A., Reid, J.L., Schork, N.J., Dominiczak, A.F., 1999. Telemetry for cardiovascular monitoring in a pharmacological study: new approaches to data analysis. Hypertension 33, 248–255.

Benigni, A., Zoja, C., Noris, M., Corna, D., Benedetti, G., Bruzzi, I., Todeschini, M., Remuzzi, G., 1999. Renoprotection by nitric oxide donor and lisinopril in the remnant kidney model. Am. J. Kidney Dis. 33, 746–753.

Calder, J.A., Schachter, M., Sever, P.S., 1992. Direct vascular actions of hydrochlorothiazide and indapamide in isolated small vessels. Eur. J. Pharmacol. 220, 19–26.

Carey, R.M., Jin, X., Wang, Z., Siragy, H.M., 2000a. Nitric oxide: a physiological mediator of the type 2 (AT2) angiotensin receptor. Acta Physiol. Scand. 168, 65–71.

Carey, R.M., Wang, Z.Q., Siragy, H.M., 2000b. Role of the angiotensin type 2 receptor in the regulation of blood pressure and renal function. Hypertension 35, 155–163.

Crespo, M.J., 1999. Vascular alterations during the development and progression of experimental heart failure. J. Card. Failure 5, 55–63.

Crespo, M.J., Escobales, N., Altieri, P.I., 1997. Altered vascular function in early stages of heart failure. J. Card. Failure 3, 311–318.

Cushman, D.W., Cheung, H.S., 1991. Concentrations of angiotensin converting enzyme activity in tissues of the rat. Biochim. Biophys. Acta 250, 261–265.

De Mello, W.C., Crespo, M.J., 1999. Correlation between changes in morphology, electrical properties, and angiotensin-converting enzyme activity in the failing heart. Eur. J. Pharmacol. 378, 187–194.

D'Uscio, L.V., Shaw, S., Barton, M., Luscher, T.F., 1998. Losartan but not verapamil inhibits angiotensin II-induced tissue endothelin-1 increase: role of blood pressure and endothelial function. Hypertension 31, 1305–1310.

Ferri, C., Desideri, G., Baldoncini, R., Bellini, C., Valenti, M., Santucci, A., De Mattia, G., 1999. Ang II increases the release of endothelin-1 from human cultured endothelial cells but does not regulate its circulating levels. Clin. Sci. (Colchester) 96, 261–270.

Fontaine, E.R., Viau, S., Jasmin, G., Dumont, L., 1998. Effects of phosphoramidon, and BQ 788 on coronary and cardiac dysfunctions on the failing hamster heart. J. Cardiovasc. Pharmacol. 32, 12–20.

Forman, R., Parmley, W.W., Sonneblick, E.H., 1972. Myocardial contractility in relation to hypertrophy and failure in myopathic Syrian hamster. J. Mol. Cell. Cardiol. 4, 203–211.

Gertz, E.W., 1972. Cardiomyopathic Syrian hamster: a possible model of human disease. Prog. Exp. Tumor Res. 16, 242–260.

Hanton, G., Barnes, P., Shepperson, N.B., Walley, R., 1993. Effects of hydrochlorothiazide and captopril on the survival and heart weight of cardiomyopathic hamsters. Res. Commun. Chem. Pathol. Pharmacol. 81, 159–166.

Haynes, W.G., Webb, D.J., 1998. Endothelin as a regulator of cardiovascular function in health and disease. J. Hypertens. 16, 1081–1098.

Israel, A., Cierco, M., Sosa, B., 2000. Angiotensin AT(2) receptors mediate vasodepressor response to footshock in rats. Role of kinins, nitric oxide and prostaglandins. Eur. J. Pharmacol. 394, 103–108.

Iyer, S.N., Yamada, K., Diz, D.I., Ferrario, C.M., Chappell, C., 2000. Evidence that prostaglandins mediate the antihypertensive actions of angiotensin-(1–7) during chronic blockade of the renin–angiotensin system. J. Cardiovasc. Pharmacol. 36, 109–117.

Kohzuki, M., Kanazawa, M., Yoshida, K., Tsutsumi, E., Yasujima, M., Abe, K., Johnston, C.I., Sato, I., 1996. Cardiomegaly and vasoactive hormones in rats with chronic myocardial infarction: long-term effects of chlorothiazide. Clin. Sci. (Colchester) 90, 31–36.

Lin, H.Y., Kaji, E.H., Winkel, G.K., Ives, H.E., Lodish, H.F., 1991. Cloning and functional expression of a vascular smooth muscle endothelin 1 receptor. Proc. Natl. Acad. Sci. 88, 3185–3189.

Liu, Y., Leri, A., Li, B., Wang, X., Cheng, W., Kajstura, J., Anversa, P., 1998. Angiotensin II stimulation in vitro induces hypertrophy of normal and postinfarcted ventricular myocytes. Circ. Res. 82, 1145–1159.

Maeso, R., Rodrigo, E., Munoz-Garcia, R., Navarro-Cid, J., Ruilope, L.M., Lahera, V., Cachofeiro, V., 1997. Losartan reduces constrictor responses to endothelin-1 and the thromboxane A2 analogue in aortic rings from spontaneously hypertensive rats: role of nitric oxide. J. Hypertens. 15, 1677–1684.

Martinez-Maldonado, M., Gely, R., Tapia, E., Benabe, J.E., 1990. Role of macula densa in diuretics-induced renin release. Hypertension 16, 261–268.

McMurray, J., McDonagh, T., Morrison, C.E., Dragie, H.J., 1993. Trends in hospitalization for health failure in Scotland 1980–1990. Eur. Heart J. 14, 1158–1162.

Noll, G., Tsuchudi, M.R., Novosel, D., Luscher, T.F., 1994. Activity of the L-arginine/nitric oxide pathway and endothelin-1 in experimental heart failure. J. Cardiovasc. Pharmacol. 23, 916–921.

Numabe, A., Uehara, Y., Hirawa, N., Takada, S., Yagi, S., 1989. Effects of thiazide diuretic on vascular eicosanoid system of spontaneously hypertensive rats. J. Hypertens. 7, 493–499.

Oparil, S., Barr, E., Elkins, M., Liss, C., Vrecenak, A., Edelman, J., 1996. Efficacy, tolerability, and effects on quality of life of losartan, alone or with hydrochlorothiazide, versus amlodipine, alone or with hydrochlorothiazide, in patients with essential hypertension. Clin. Ther. 18, 608–625.

Opsahl, J.A., Goldberg, M.R., Katz, S.A., 1995. Effect of acute and chronic losartan therapy on active and inactive renin and active renin glycoforms. Am. J. Hypertens. 8 (11), 1090–1098.

Oriji, G.K., 1999. Angiotensin II-induced ET and PGI2 release in rat aortic endothelial cells is mediated by PKC. Prostaglandins, Leukotrienes Essent. Fatty Acids 61, 113–117.

Pitt, B., Poole-Wilson, P.A., Segal, R., Martinez, F.A., Dickstein, K., Camm, A.J., Konstam, M.A., Riegger, G., Klinger, G.H., Neaton, J., Sharma, D., 2000. Effect of losartan compared with captopril on mortality in patients with symptomatic heart failure: randomized trial —the Losartan Heart Failure Survival Study ELITE II. Lancet 355, 1582–1587.

Richer, C., Domergue, V., Vincent, M.P., Giudicelli, J.F., 1996. Involvement of nitric oxide, but not prostaglandins, in the vascular sympathoinhibitory effects of losartan in the pithed spontaneously hypertensive rat. Br. J. Pharmacol. 117, 315–324.

Rizzoni, D., Porteri, E., Piccoli, A., Castellano, M., Bettoni, G., Muiesan, M.L., Pasini, G., Guelfi, D., Mulvany, M.J., Agabiti, R.E., 1998. Effects of losartan and enalapril on small artery structure in hypertensive rats. Hypertension 32, 305–310.

Rodrigo, E., Maeso, R., Munoz-Garcia, R., Navarro-Cid, J., Ruilope, L.M., Cachofeiro, V., Lahera, V., 1997. Endothelial dysfunction in spontaneously hypertensive rats: consequences of chronic treatment with losartan or captopril. J. Hypertens. 15, 613–618.

Ruiz-Opazo, N., Hirayama, K., Akimoto, K., Herrera, V.L., 1998. Molecular characterization of a dual endothelin-1/Ang II receptor. Mol. Med. 4, 96–108.

Schieffer, B., Wirger, A., Meybrunn, M., Seitz, S., Holtz, J., Riede, U.N., Drexler, H., 1994. Comparative effects of chronic angiotensin-converting enzyme inhibition and angiotensin II type 1 receptor blockade on cardiac remodeling after myocardial infarction in the rat. Circulation 89, 2273–2282.

Schmect, J., Krafft, P., Groschler, A., Heller, A., Neuhof, H., van Ackern, K., Koch, T., 1999. Characterization and distribution of endothelin receptors in the pulmonary circulation: investigation of isolated, perfused, and ventilated rabbit lungs. Shock 12, 247–254.

Strobeck, J.E., Factor, S.M., Bhan, A., Sole, M., Liew, C.C., Fein, F., Sonnenblick, E.H., 1979. Hereditary and acquired cardiomyopathies in experimental animals; mechanical, biochemical and structural features. Ann. N.Y. Acad. Sci. 317, 58–88.

Tracey, W.R., Tse, J., Carter, G., 1995. Lipopolysaccharide-induced changes in plasma nitrite and nitrate concentrations in rats and mice: pharmacological evaluation of nitric oxide synthase inhibitors. J. Pharmacol. Exp. Ther. 272, 1011–1015.

Traub, Y.M., Nemes, L.M., Aloof, S., Rosenfeld, J.B., 1976. Stimulation of renin secretion by various methods: optional results with hydrochlorothiazide. Int. Urol. Nephrol. 8, 247–255.

Tripodi, F., Stanke-Labesque, F., Devillier, P., Chavanon, O., Sessa, C., Bessard, G., 1999. Antagonistic effects of losartan on thromboxane A2-receptors in human isolated gastroepiploic artery and saphenous vein. Cardiovasc. Pharmacol. 34, 734–740.

Tsutsui, H., Tomoike, H., Nakamura, M., 1990. Quantitative and autoradiographic analyses of alpha-adrenergic and serotonergic receptors on aorta and coronary artery. Am. J. Physiol. 259 (5 Pt. 2), H1343–H1350.