Distribution of the activity of the angiotensin-converting enzyme in the rat aorta and changes in the activity with aging and by the action of L-NAME

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Abstract The activity of the angiotensin-converting enzyme (ACE) of the inner surface (the endothelium surface) of rat aorta sections has been studied depending on their distance from the aortic arch, age of rats, and the duration of treatment of rats with the NO synthase inhibitor, N_{ω} -nitro-L-arginine (L-NAME). The activity of ACE of aorta sections was determined by measuring the hydrolysis of hippuryl-L-histidyl-L-leucine and was expressed as picomoles of Hip-His-Leu hydrolyzed per minute per square millimeter of the endothelium surface. It was found that the ACE activity considerably varies along the aorta of young rats. This variability decreases with increasing age of rats and by the action of L-NAME. The average ACE activity in the aorta increases with the age of rats and with increasing time of L-NAME treatment. Enalapril normalizes the distribution of the ACE activity along the aorta and decreases the average ACE activity. The changes in the distribution of the ACE activity along the aorta and in the average ACE activity in the aorta

with increasing age of the rat and by the action of L-NAME may play a role in the development of atherosclerosis of vessels on aging and the inhibition of formation of nitric oxide.

Keywords Aging · Angiotensin-converting enzyme · Aorta · L-NAME

Introduction

The angiotensin-converting enzyme (ACE), which transforms angiotensin I into the pressor angiotensin II and destroys the depressor bradykinin, is one of the main regulators of the vascular tone and blood pressure. It is known that the pressure increases with aging (Basso et al. 2007) and by the action of the NO synthase inhibitor N_{ω} -nitro-L-arginine (L-NAME) (Pollock et al. 1993; Takemoto et al. 1997b). In addition, by the action of these factors there occur vascular changes indicating the development of atherosclerosis (Basso et al. 2007; Katoh et al. 2001; Takemoto et al. 1997b). The inhibitors of ACE suppress the development of pathological changes in vessels. Thus, they decrease the blood pressure, restore the level of endothelial NO synthase (eNOS) and the response of vessels to acetylcholine, prevent the age-related thickening of the aorta and the accumulation of collagen in it (Albaladejo et al. 1994; Basso et al. 2007; De Gennaro et al. 2005; Katoh et al. 2001; Michel et al. 1994; Pollock et al.

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1993; Takemoto et al. 1997a;), and even increase the life of rats (Basso et al. 2007). The data discussed above indicate that the vascular pathology caused by aging and the blockade of NO synthase is related to the activity of ACE. However, the question concerning the changes in the ACE activity in rat vessels depending on age and by the action of L-NAME is not yet completely understood. Mooradian and Lieberman studied the ACE activity in blood plasma of rats over a long period of their life, from 2 to 25 months, and found that it decreased with increasing age (Mooradian and Lieberman 1990). Therefore, the age-dependent increase in blood pressure and the development of vessel pathology do not correlate with changes in the ACE activity in the blood plasma. We are familiar only with one study devoted to the ACE activity in the aorta of aged (10-, 20-, and 30-month-old) rats (Challah et al. 1997). It was shown that the ACE activity in the aorta by month 30 increased by 14% compared with that by month 10. In this case, the ACE activity in the blood plasma also decreased, as was found by Mooradian and Lieberman (1990). Changes in the ACE activity in the aorta, blood plasma, and other tissues of rats by the action of L-NAME were studied by Takemoto et al. (1997b) and Sharifi et al. (2005). It was shown that the ACE activity increased only in the heart and aorta. In the aorta, it increased 1.4 times after 1 week of treatment with L-NAME, three times after 4 weeks of treatment, and four times after 8 weeks of intake of L-NAME (Takemoto et al. 1997b). The ACE activity was examined just at the time when pathological changes developed in the aorta. It should be noted that the ACE activity was determined, as in work of Challah et al. (1997), in a homogenate of the aorta. However, it is known that the ACE activity depends on the concentration of NO and cyclic GMP (Linz et al. 1999) and can vary due to the interaction of ACE with the receptor of kinin B₂ (Sabatini et al. 2008). When the ACE activity is measured in homogenates, all factors regulating the ACE activity in the tissue are eliminated; i.e., factually not the activity of the enzyme but its amount in tissues is determined.

Besides, the ACE activity is localized predominantly on the surface of endothelial cells (Fleming 2006; Paul et al. 2006; Zhuo et al. 1998), and the calculation of ACE activity per aorta weight under-

estimates its value the more the smaller is the weight portion of endothelium in a vessel. The weight portion of the endothelium relative to the whole aorta weight strongly decreases on aging and after L-NAME treatment since the content of elastin and collagen in the vessel wall increases, and hence the distortion of ACE activity in these cases would be more pronounced. There is evidence that different aorta parts differ by their contractile responses to angiotensin II and the expression of receptors. Contractile responses to angiotensin II were more pronounced in abdominal than thoracic aortic ring segments from male C57BL/6 mice (Zhou et al. 2003), and the amount of AT1aR mRNA in the abdominal aorta was much greater compared to the thoracic aorta of male mice (Henriques et al. 2008; Zhou et al. 2003). Different reactions of thoracic and abdominal aorta segments were shown in vitro where angiotensin II concentration was the same for different segments (Zhou et al. 2003). The local concentration of angiotensin II throughout the aorta may vary in vivo, and the main cause of this variability may be a difference in the ACE activity. This means that the contractile response of aorta parts in vivo is determined by not only receptor expression but also the ACE activity. The variability in the ACE activity along the aorta has not yet been measured.

In this work, we studied the ACE activity of the inner surface of the aorta, predominantly of its endothelium. This approach corresponds better to the in vivo conditions and makes it possible to examine both the average ACE activity in the aorta and its distribution along the aorta. Changes in the ACE activity with age and at early times of treatment with L-NAME (up to 2 weeks) were also studied.

Materials and methods

Animals and aorta preparation

Male Wistar rats weighing 200–500 g at an age of 4–60 weeks (animal collection of the Institute of Theoretical and Experimental Biophysics, Pushchino, Russia) were used. Rats ($N=119$; 76 ten-week-old rats and 43 rats of another age) were maintained in animal facilities with free access to water and standard rat chow (control group). The local ethics committee

criteria for care and use of laboratory animals were carefully followed. The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). The animals of experimental groups were given L-NAME (1 mg/ml, 4–12 days) or/and the ACE inhibitor enalapril maleate (0.1 mg/ml, 8 days) dissolved in drinking water. When L-NAME was given in combination with enalapril, these substances were simultaneously added to drinking water. Routine monitoring showed that rats consumed approximately 100 ml of drinking water per 1 kg of body weight each day irrespective of whether or not L-NAME was contained in water, and the drinking regime did not change throughout all treatment protocols. At this rate of water drinking, each rat received daily 100 mg of L-NAME or/and 10 mg of enalapril per kg body weight. The aorta was prepared according to Korystov et al. (2009). Before operation, animals were anesthetized by ether. Then the thorax was opened, and heparin (500 units) was injected into the heart to prevent blood clotting. The procedure from the beginning of operation to the removal of the aorta took less than 3 min. Rats were under anesthesia during this period and died shortly after the injection of heparin. A greater part of adventitial fat adherent to the aorta was cleaned in situ. Then the aorta was removed, rinsed with cold (4°C) 10 mM Hanks–HEPES solution, pH 7.4, and placed in the same solution. Residuary fat was carefully cleaned; care was taken not to damage the endothelium. The aorta was cut beginning from the aortic arch into eight 4- to 5-mm sections. The point at which the aorta became parallel with the vertebral column was taken as the end of the aortic arch. Sections 1–5 were in the thorax aorta, and sections 7 and 8 were in abdominal aorta irrespective of the age of rats. Section 6 was in thorax aorta part in 10-week-old rats and in the abdominal aorta part in 40-week-old or elder rats. An aortic section 4–5 mm long (4.4 mm on average) had a wet weight of 2–4.7 mg depending on the age of the rat. The aortic sections were numbered from 1 to 8 starting with the section adjacent to the aortic arch. Aortic sections were cut lengthwise, turned inside out with the endothelium outside, and attached to the tip of a plastic pipette with a polyester thread. After the measurement of the ACE activity, aortic sections were taken away from

the pipette, flattened, and their linear dimensions were determined using a slide gauge to an accuracy of 0.1 mm.

Measurement of ACE activity in the whole aorta

The ACE activity was determined by measuring the hydrolysis of hippuryl–L-histidyl–L-leucine (Hip–His–Leu) using the method described by Ackermann et al. (1998) with a modification of Miyamoto et al. (2002). Briefly, isolated rat aorta sections were placed in Hanks–HEPES solution, pH 7.4, (450 μ l) and incubated for 10 min at 37°C with shaking (25 Hz, amplitude 1 mm) for adaptation before the addition of the ACE substrate. The reaction was started by the addition of 10 mM Hip–His–Leu (50 μ l). After 30 min of incubation at 37°C, the reaction was stopped by the addition of 1000 μ l of 0.1 N NaOH. The reaction mixture was stirred, the aorta sections were taken out of the solution, and their dimensions and weight were determined. A 200- μ l aliquot of the remaining solution was incubated with 50 μ l of *o*-phthaldialdehyde (2 mg/ml in dimethyl sulfoxide) for 30 min at 37°C, and the reaction was stopped by the addition of 2 ml of 0.8 N HCl. The samples were centrifuged at 3,000 $\times g$ at 4°C for 5 min, and fluorescence was measured using an MF44 Perkin-Elmer fluorimeter at excitation and emission wavelengths of 360 and 500 nm, respectively. For determining the ACE activity, a standard curve was generated using His–Leu. The ACE activity was expressed as picomoles of Hip–His–Leu hydrolyzed per minute per square millimeter of the inner aorta surface (the endothelium surface) or per mg of wet tissue ($\text{pmol min}^{-1} \text{mm}^{-2}$ or $\text{pmol min}^{-1} \text{mg}^{-1}$). The average ACE activity in the aorta was determined by averaging the ACE activities of all (eight) sections for each rat, and then these values were averaged for all rats used in the experiment.

Measurement of the ACE activity in the denuded aorta

To assess the endothelial ACE activity, the endothelium was removed by gently rubbing the surface of the aorta intima with the closed tips of hemostatic forceps as previously described (Ohara et al. 1993). Four 10-week-old rats were used to determine the ACE activity in the denuded aorta. All procedures

with denuded aortic sections and the averaging of the results were the same as for whole aortic sections.

Drugs

*N*_ω-nitro-L-arginine methyl ester, enalapril maleate, dimethyl sulfoxide, Hank's solution, HEPES, Hip-His-Leu acetate salt, His-Leu, and *o*-phthalaldehyde were obtained from Sigma (USA). Heparin was a pharmaceutical preparation.

Statistical analysis

The results are expressed as means±SEM. The numbers of rats (*N*) used in experiments are given either in figure legends or in the text. The significance of differences in multiple comparisons was determined by ANOVA and Tukey's test. *P* values less than 0.05 were considered significant.

Results

Dependence of the average ACE activity in the aorta on the age of rats and L-NAME treatment

Figure 1 shows changes in the average ACE activity with age, expressed in pmol min⁻¹ mm⁻² (a) and pmol min⁻¹ mg⁻¹ (b). A comparison of Fig. 1a and b shows that the dependence of the ACE activity on age

is substantially distorted if the activity is calculated per unit wet weight. This can be explained by the fact that the weight of sections increases with age mainly at the expense of the thickness of the aorta, due to the accumulation of elastin and collagen, which do not contribute to the ACE activity; hence upon calculation per unit weight, the underestimation of the ACE activity increases with age. The ACE activity expressed per unit of the aorta inner surface changes little over a period of 10 weeks and sharply increases with further increase in the age. The gain in the activity is about 38% per each subsequent doubling of age relative to 10-week-old rats.

Figure 2 shows how the intake of L-NAME affects the average ACE activity in the aorta. It is seen that the ACE activity increases with the duration of L-NAME treatment; after 12 days, the gain in the activity reaches 80%.

Distribution of the ACE activity along the aorta length in young and old control rats: effect of L-NAME and enalapril

As indicated in the “Materials and methods” section, the inner surface of the aorta was exposed to the solution containing the ACE substrate; hence, we determined the ACE activity mainly in the endothelium of the vessel. Therefore, the ACE activity was calculated per unit area of the inner surface of the aorta. That endothelium makes the main contribution

Fig. 1 Dependence of the average ACE activity in the aorta on the age of rats, as calculated per inner surface area (a) or wet weight (b). *N*=6–7 for each time point

Fig. 2 Dependence of the average ACE activity in the aorta on the time of consumption of L-NAME by rats. The age of rats at the beginning of the experiment was 10 weeks. $N=6-9$ for each time point

to the aortic ACE activity is supported by a sharp decrease in the ACE activity after the removal of endothelium, from $20.9 \pm 2.0 \text{ pmol min}^{-1} \text{ mm}^{-2}$ in the native aorta ($N=6$) to $7.3 \pm 0.4 \text{ pmol min}^{-1} \text{ mm}^{-2}$ in the denuded aorta ($N=4$) of 10-week-old rats.

It is known that the diameter of the aorta decreases with increasing separation of the aorta from the heart and increases with the age of rats (Berry et al. 1972). Figure 3 shows relative changes in the inner

circumference (L) and the area of the inner surface (S) of a 4.4-mm aortic section for the rats of different age (10–40 weeks) depending on the distance of the section from the aortic arch (as compared to the first section of the aorta). It is shown in Fig. 3 that the area of the inner surface of the aorta decreases with increasing separation from the heart. Figure 4 shows an increase in L_m (a), S_m (b), and the wet weight (c) of sections with increasing age of rats. The data

Fig. 3 Changes (relative to section no. 1) in **a** the length of the inner circumference (L) and **b** inner surface (S) of aorta sections depending on the separation from the aorta arch. $N=24$

Fig. 4 Changes in **a** the average length of the inner circumference (L_m), **b** the area of the inner surface (S_m) and **c** the wet weight of aorta sections depending on the age of rats. $N=20$. SEMs are less than the symbol size

presented in Figs. 3 and 4 were used to calculate the ACE activity either per unit area of the inner surface of the aorta or per unit of wet weight.

Figure 5 shows the absolute values of the ACE activity (Fig. 5a) and its distribution along the aorta length (Fig. 5b) per unit area of the inner surface of the aorta in 10-week-old control rats (1), rats receiving L-NAME for 6 (2) and 12 (3) days, and in 52-week-old control rats (4). It is seen from Fig. 5a that the ACE activity increases with the duration of L-NAME treatment and the age of rats, and the distribution of ACE activity along the aorta also changes (Fig. 5b). The ACE activity in sections 2, 3, and 4 in 10-week-old control rats is by 20–25% higher than that in the first section; then it decreases with increasing separation from the aortic arch to reach about 70% in section 8. The ACE activity in sections 2–4 relative to that in section 1 decreases with both the age of rats and the time of treatment with L-NAME. In rats receiving L-NAME for 12 days

and in 52-week-old rats, the ACE activity only decreases with increasing separation from the aortic arch. Thus, the variability of the ACE activity along the aorta decreases with the duration of L-NAME treatment and with the age of rats.

The ACE inhibitor enalapril at a dose of 10 mg/kg body weight suppresses the average ACE activity in control 10- and 47-week-old rats and in 10-week-old rats receiving L-NAME for 8 days approximately to an equal level, $6.7 \pm 0.3 \text{ pmol min}^{-1} \text{ mm}^{-2}$ (see Fig. 6a). Enalapril decreases the ACE activity 3.1 times in control 10-week-old rats, 4.3 times in rats receiving L-NAME, and 5.1 times in control 47-week-old rats. Figure 6b shows that enalapril does not affect the distribution of the ACE activity in 10-week-old control rats. In 10-week-old rats receiving enalapril and L-NAME for 8 days and in 47-week-old rats receiving enalapril alone, the distribution of the ACE activity along the aorta was similar to that in the control 10-week-old rats, i.e., enalapril caused an increase in the ACE activity in sections 2–4 relative to section 1 followed by a progressive decrease with the distance along the aorta up to section 8.

Discussion

The activity of ACE of the endothelium surface of rat aortic sections was studied depending on their distance from the aortic arch, age of rats, and the duration of treatment of rats with the NO synthase inhibitor L-NAME. The major findings of this study are as follows: (a) the ACE activity considerably varies along the aorta of young rats; (b) this variability decreases with increasing age of rats and by the action of L-NAME; (c) the average ACE activity in the aorta increases with the age of rats; (d) enalapril normalizes the distribution of the ACE activity along the aorta and decreases the average ACE activity.

The experiment with the denuded aorta shows that the main contribution (65%) to the aortic ACE activity is made by the endothelium. The 35% contribution of the subendothelial part of the aorta to the aortic ACE activity, as determined in this experiment, may be overestimated because the endothelium scavenges the ACE substrate in the native aorta, and hence the ACE activity of the subendothelial layer must be smaller than without the endothelium.

Fig. 5 **a** Absolute and **b** relative (relative to that in section no. 1) ACE activity of aorta sections depending on the separation from the aorta arch. 1 control 10-week-old rats, 2 10-week-old rats receiving L-NAME for 6 days, 3 control 52-week-old rats, 4 10-week-old rats receiving L-NAME for 12 days. $N=6-12$. In **(a)** $*P<0.05$ vs. the first section in curve 1; in **(b)** $*P<0.05$ for vs. the first sections; the second sections in curves 3 and 4 do not differ from the first sections; $**P<0.05$ vs. the second sections for all curves

The average aortic ACE activity determined in the present study per milligram of wet weight (Fig. 1b) is higher than that determined in aorta homogenates (Challah et al. 1997; Takemoto et al. 1997b). Takemoto et al. (1997b) did not indicate in the Methods section whether the homogenates were centrifuged; it is likely that the ACE activity was determined in the whole homogenate. It was shown that the ACE activity in an aorta homogenate of 21- to 29-week-old rats is about

6 nmol h⁻¹ mg⁻¹ (about 100 pmol min⁻¹ mg⁻¹) and is approximately the same for the rats of this age. Our data show that the average ACE activity in the aorta of rats of this age is about 200 pmol min⁻¹ mg⁻¹, i.e., two times higher. In the work of Challah et al. (1997), the aorta homogenate was centrifuged at 1,000×g for 15 min, and the ACE activity was determined in the supernatant per mg of protein. The ACE activity in the aorta of 10-month-old rats was 21 pmol min⁻¹ mg

Fig. 6 **a** Absolute and **b** relative (relative to that in section no. 1) ACE activity of aorta sections depending on the separation from the aorta arch in control young rats and after enalapril treatment. 1 10-week-old rats, 2 10-week-old rats receiving enalapril for 8 days, 3 47-week-old rats receiving enalapril for 8 days, 4 10-week-old rats receiving enalapril+L-NAME for 8 days. $N=3-4$. In **(a)**, $*P<0.05$ vs. the first section in curve 1; in **(b)** $*P<0.05$ vs. the first sections for all curves; $**P<0.05$ vs. the second sections for all curves

protein⁻¹. Assuming that the protein accounts for only 20% of the wet weight of the tissue, this value corresponds to 4 pmol min⁻¹ mg⁻¹ of wet tissue, which is 25 times less than the value obtained by Takemoto et al. (1997b). This is probably due to the loss of a greater amount of ACE, which as a membrane-bound protein is sedimented with large tissue fragments during centrifugation. The lower value of the ACE activity obtained by Takemoto et al. (1997b) for the whole aorta extract, as compared with our data, may be due to both the disturbance of the tissue regulation of the ACE activity on homogenization and the different amounts of liquid adsorbed on the aorta surface before weight determination.

The ACE activity increases with the duration of intake of L-NAME by rats (Fig. 2). The change in the ACE activity caused by L-NAME is consistent with the data obtained by Takemoto et al. (1997b), who showed that the ACE activity after 7 days of intake of L-NAME increases by 40%. In our experiments, the ACE activity after 8 days of L-NAME intake increased by 37%.

We studied the ACE activity in the endothelium of the aorta depending on the age of rats in the range of 4–60 weeks (Fig. 1a). As it is seen, the ACE activity increases with age, the gain in the activity sharply increasing in rats aged more than 10 weeks. It is known that, beginning with this age, the growth of both the body and the aorta of the rat sharply decreases (Khan et al. 2002). The slowing down of the growth of rats after 10 weeks is evidently due to a decrease in the level of the growth hormone, which may also be the cause of the increase in the ACE activity in the aorta. Indeed, the production of the growth hormone decreases with the age of the rat (San Frutos et al. 2007; Sontag et al. 1980), and the ACE activity depends on the growth hormone. It was reported that the enhanced ACE activity in the heart of senescent rats decreases after the administration of the growth hormone (Groban et al. 2006).

It was shown earlier that hypertension and changes in vessel structure were associated with an increase in the aortic ACE after L-NAME treatment (Sharifi et al. 2005; Takemoto et al. 1997b). It is known that the blood pressure and vascular structural changes increase with aging also (Basso et al. 2007). The age-related increase in the ACE activity revealed in our work can be also the cause of functional and structural changes of vessels as in the case of L-NAME treatment.

We showed that enalapril at a concentration of 0.1 mg/ml taken in with drinking water decreases the ACE activity in the aorta of rats of different groups to a similar level (Fig. 6a), which amounts to 32% of the activity in control 10-week-old rats, 23% of the activity in rats receiving L-NAME, and 20% of the ACE activity in 47-week-old rats. In the work of Takemoto et al. (1997a), temokapril (0.1 mg/ml taken in with drinking water) lowered the ACE activity in the heart of rats receiving L-NAME to 19% of the level registered without the intake of the ACE inhibitor, which amounted to 44% of the ACE activity in control rats. The effect of the ACE inhibitor imidapril (0.2 mg/ml taken in with drinking water) on the ACE activity in the heart of rats receiving L-NAME was also studied by Katoh et al. (2001). It was shown that the inhibitor at this dose lowers the ACE activity to 10% of the value for rats receiving L-NAME and to 30% of the activity level in the hearts of control rats. A comparison of the results reported by Takemoto et al. (1997a) and Katoh et al. (2001) shows that the application of a higher dose of the inhibitor leads to a decrease in the residual ACE activity. Presumably, the hydrolysis of Hip–His–Leu in the aorta sections of rats receiving enalapril at a dose of 0.1 mg/ml (in water) is due partially to an incomplete suppression of ACE. Chimase, which increases the formation of angiotensin II in vessels upon the inhibition of ACE (Doggrell and Wanstall 2004), may also contribute to the residual hydrolysis of Hip–His–Leu.

The variability of the ACE activity along the aorta length and changes in the variability by the action of the NO synthase inhibitor and with increasing age of the rat have been shown for the first time (Figs. 5 and 6). A possible reason for the variability of the ACE activity in the aorta of young rats may be the different shear stress along the aorta length during blood movement because the ACE activity depends on shear stress (Rieder et al. 1997). It is known that the blood pressure increases with age and the duration of L-NAME treatment (Basso et al. 2007; Pollock et al. 1993; Takemoto et al. 1997b). The variability of the ACE activity decreases as the blood pressure increases with rat age and by the action of L-NAME (Fig. 5) and is restored under the influence of enalapril (Fig. 6), which decreases the blood pressure to the normal level at a dose of 10 mg/kg of body weight (Pollock et al. 1993). It is possible that

the increase in blood pressure smoothes the shear stress in the aorta. The restoration of the normal distribution of the ACE activity along the vessels may play a role in the antiatherogenic effect of ACE inhibitors. There are literature data indicating that aorta segments differ in many properties. Some of them are mentioned in Introduction: the magnitude of contractile response to angiotensin II (Zhou et al. 2003) and expression of AT1a receptors (Henriques et al. 2008) in thoracic and abdominal aorta segments. Furthermore, muscle cells of thoracic aorta segments differ from those of abdominal segments by origin (Majesky 2007), contractile response to hydrogen peroxide (Ardanaz et al. 2008), noradrenaline, and endothelin (Dhein et al. 2001), by the rate of inositolphosphate formation and Ca^{2+} transport, as well as the amount of Ca^{2+} -ATPase mRNA (Levitsky et al. 1993), the content of integrin (Winter et al. 2003), and the distribution of aortic foam cells in hyperlipidemic rabbits (Ruehm et al. 2001). These and our data on ACE activity distribution in the aorta are necessary for studying the mechanism of atherosclerosis since it is more pronounced in the human abdominal aorta, the fact that was noted by pathologists many years ago (Boyd 1947).

Concerning the reasons of changes in the average ACE activity and its variability along the aorta, we believe that the change in NO production is a more likely mechanism. It was shown that ACE activity and ACE expression depend on the production of NO (Linz et al. 1999). A chronic inhibition of eNOS led to an upregulation of cardiac and vascular ACE activity. In contrast, it was demonstrated that NO or NO-releasing compounds inhibit the activity of purified ACE in a concentration-dependent and competitive manner. Linz et al. concluded in their review: “Taken together, these observations provided evidence for a ‘cross talk’ between eNOS expression/activity and tissue ACE expression/activity by means of a feedback regulation.” Therefore, the increase of the average ACE activity in aorta with age and upon the inhibition of NO synthase (L-NAME treatment) can be caused by a decrease in NO production. It is known that the amount of eNOS (Csiszar et al. 2002) and NO bioavailability (Hamilton et al. 2001) decrease with age. The differences in the ACE activity along the aorta can also be due to the variability of NO synthase activity because it was reported that shear stress, which varies along the

aorta, stimulates the production of endothelial NO (Milner et al. 1990).

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