Journal of Andrology, Vol. 26, No. 1, January/February 2005

Restoration of Erectile Capacity in Normotensive Aged Rats by Modulation of Angiotensin Receptor Type 1

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ABSTRACT: We investigated the effects of systemic modulation of angiotensin 2 on the erectile dysfunction of aged rats. Young and aged (18 months old) male Sprague-Dawley rats were either treated with daily administration of losartan (angiotensin type 1 receptor antagonist, 30 mg/kg/d PO) or the drug vehicle (control) for 4 weeks (n = 6 in each group). We monitored the intracavernosal pressure (ICP) after administration of apomorphine (100 μg/kg), and we measured the degree of lipid peroxidation of corpus cavernosum and the cavernosal protein expression by an immunoblot technique. Compared to the control young rats, the control aged rats showed significant impairment of erectile function; however, losartan treatment effectively restored the erectile function of aged rat to a level similar to that of young rats. Despite the systemic pressure-lowering effect of the drug, the peak ICP was not significantly reduced; rather, the ICP/systemic arterial pressure (SAP) was increased by the losartan treatment. Measurement of lipid peroxidation revealed the fact that the drug was effective in diminishing oxidative stress. While the losartan treatment significantly enhanced the expression of endothelial nitric oxide synthase (eNOS), it had no effect on the expression of transforming growth factor (TGF)-β1. The results obtained indicated that alteration of the renin-angiotensin system might be implicated in the erectile dysfunction of elderly males, and modulation of this system may be of great therapeutic value.

Key words: Erectile dysfunction, Sprague-Dawley rat, losartan.
J Androl 2005;26:123–128

Aging men often suffer from erectile dysfunction, and according to the Massachusetts Male Aging study, the percentage of potent men between the ages of 40 and 70 years will decrease from 60% to 33% (Feldman et al, 1994). Although the causes of this age-related erectile dysfunction are not well understood, age-related systemic arterial insufficiency (Tarcan et al, 1998) and structural and functional alterations of the aging penis (Seftel, 2003) have been suggested as the probable causes.

It is well known that aging is associated with diminished aortic compliance and resting cardiac output. In addition, such structural changes as intimal-medial thickening and increased arterial stiffness are usually associated with vascular aging (Lakatta and Levy, 2003), and this may contribute to the increased incidence of target organ changes such as nephrosclerosis, left ventricular hypertrophy, and decreased aortic compliance. In the penis, a decreasing trend in peak systolic velocity with aging has also been observed (Chung et al, 1997).

Structural and functional alterations of the aging penis consist of endothelial dysfunction (Cartledge et al, 2001), up-regulation of corporal smooth muscle tone (Christ et al, 1991), diminished cavernosal smooth muscle and elastic fibers, and an increase of cavernosal fibrosis (Wespes, 2002). The derangement of nitric oxide (NO) metabolism and the increased expression of transforming growth factor β1 (TGF-β1) may be implicated in these changes (Dahiya et al, 1999; Rajasekaran et al, 2002).

Angiotensin II (Ang II) is the main active metabolite of the renin-angiotensin system. It has been shown that physiological amounts of Ang II are produced and its receptors have been identified in the corpus cavernosum (Kifor et al, 1997). Therefore, Ang II and its receptors are thought to play a role in the regulation of cavernosal tone. For example, an intracavernosal injection of Ang II has been reported to terminate erection in anesthetized dogs (Kifor et al, 1997), and Ang II is thought to play a role in penile detumescence (Becker et al, 2001). Furthermore, Ang II regulates the vascular tone by counteracting the effects of NO, the principal mediator of penile erection.

Based on these research findings, there is speculation as to whether disturbances in the systemic or local secretion or degradation of Ang II may contribute to the development of male erectile dysfunction. In other vascular beds, recent studies (Kansui et al, 2002; Weber, 2002) have shown that the role of Ang II was not limited to local regulation of vascular smooth muscle tone; rather,

Supported by grant 03-2003-004-0 from the SNUH Research Fund.
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Received for publication June 12, 2004; accepted for publication September 9, 2004.

it is also believed to play an important role in the cardiovascular pathophysiology, such as endothelial damage, cell proliferation, vascular remodeling, and oxidation. Therefore, drugs that antagonize the effects of Ang II, such as angiotensin converting enzyme inhibitor (ACEI) or angiotensin type 1 receptor (AT1) antagonist, have been reported to potentially prevent age-related endothelial dysfunction even in normotensive patients (Devereux et al, 2003).

We have hypothesized that Ang II antagonism would be helpful to correct age-related erectile dysfunction even if the erectile dysfunction is not associated with hypertension, and erectile function may be assisted by the improvement of the local penile molecular environment as well as the systemic blood flow.

To demonstrate our hypothesis, we examined the effects of AT1 antagonist on erectile function and on relevant molecular environment in the penises of aged normotensive rats.

Materials and Methods

Experimental Animals

Twelve young adult (3-month-old) and 12 aged (18-month-old) male Sprague-Dawley rats were purchased from a commercial source, and these rats were maintained on a 12-hour light-dark cycle, with food and water being made available ad libitum. All the experimental procedures we performed were approved by the Institutional Animal Care and Use Committee of Seoul National University.

Drug Treatments

Young and aged animals were assigned to the control or treatment groups (n = 6 in each group). The control animals had free access to water and standard rat chow. The treated animals received the AT1 antagonist losartan (Cozaar™, MSD, Seoul, Korea) (30 mg/kg/d) in their drinking water for 1 month. The concentration of the losartan contained in the drinking water was determined based on the rats' previously established drinking patterns.

In Vivo Evaluation of Erectile Function

The rats were anesthetized with an intraperitoneal injection of urethane (1.6 g/kg), and they were placed on a homeothermic blanket maintained at 37°C. The rats' left carotid artery was cannulated for the continuous monitoring of the mean arterial blood pressure. The right corpus cavernosum was cannulated to permit continuous monitoring of intracavernosal pressure (ICP). Pressure data were collected and analyzed electronically (Powerlab™, ADInstruments, Colorado Springs, Colo).

A stabilizing period of 20–30 minutes was allowed before recording of ICP and systemic arterial pressure (SAP). Apomorphine 100 μ/kg was given subcutaneously, and then erectile function was assessed by simultaneous SAP and ICP recordings for 30 minutes. The time to the first peak pressure, the maximal ICP, the mean number of ICP increases, the area under curve (AUC), and the maximal ICP/mean SAP were analyzed for each rat. The statistical analysis was then performed for all the animals included in each experimental group.

At the completion of the given observation periods, the animals were euthanized by an overdose of phenobarbital. The rats' penises were immediately removed, cleaned, frozen in liquid nitrogen, and stored at −70°C until the time of further processing.

Immunoblotting

The preparation of the penile tissue for immunoblotting was performed as previously described (Akingba and Burnett, 2001). Homogenized penile tissue was centrifuged at 14 000 × *g* for 20 minutes at 4°C. The protein concentration was determined with BCA Protein Assay reagents (Pierce Chemical Company, Rockford, Ill) according to manufacturer protocol. An equal amount (20 mg) of each sample was diluted and fractionated onto 15% sodium dodecyl sulfate–polyacrylamide gel for electrophoresis. After electrophoresis, the proteins were then transferred to a Hybond-ECL (Amersham Life Sciences, Ghent, Belgium) nitrocellulose membrane; they were blocked for 1 hour with blotto-Tween (5% nonfat dry milk and 0.1% Tween-20) and then incubated for 1 hour at room temperature in a 1:2000 dilution of mouse anti–TGF-β1 or mouse anti-endothelial nitric oxide synthase antibody (Transduction Laboratories, Lexington, Ky) in 5% nonfat dry milk. The bound antibody was detected with labeled goat anti-rabbit immunoglobulin G secondary antibody conjugated to horseradish peroxidase, and the results of this reaction were visualized using enhanced chemiluminescence.

The eNOS and TGF-β1 proteins corresponded to a 145-kd band and a 42-kd band, respectively. The density of respective bands was quantified by densitometric scanning of the Western blots using computer software (Media Cybernetics, San Diego, Calif).

Determination of Lipid Peroxidation by Reactive Oxygen Species

We used an MDA-586 colorimetric assay kit according to the manufacturer's instructions (OXIS International, Portland, Oreg), which quantitatively measured the product malondialdehyde (MDA) of the lipid peroxidative reaction to assess the lipid peroxidation in the penile tissue homogenates. Briefly, the samples were first homogenized in ice-cold 20 mM Tris-HCl buffer (pH 7.4). After centrifugation at 3000 × *g* at 4°C for 20 minutes, an aliquot of 200 μl of the supernatant was then used to detect a stable chromophore that was produced by the reaction of a chromogenic reagent with MDA in the supernatant at 45°C for 60 minutes. The absorbance of this chromophore was measured at 586 nm on a microtiter plate reader (Dynex Technologies, Chantily, Va). The degree of lipid peroxidation was determined by a MDA standard curve as expressed by malondialdehyde equivalent content (nmol MDA/mL).

Statistical Analysis

Measured data were expressed as means plus or minus standard error of the mean. One-way analysis of variance (ANOVA) or the Mann-Whitney *U* test using SPSS Windows (version 11) was used for comparisons between groups with regard to the drug or

Table 1. *Erectile parameters of apomorphine-induced erection**

	CA	CY	TA	TY	*P*†	*P*†‡
Time to first response (s)	352 ± 22	294 ± 21	292 ± 16	278 ± 31	.01	.18
No. of erection	2.9 ± 1.1	3.2 ± 0.6	4.2 ± 1.5	4.5 ± 0.5	.21	.11
Max ICP (mmHg)	42.8 ± 7	65.4 ± 5	60.5 ± 5	68.8 ± 12	.01	.52
AUC (mmHg/s)	434 ± 122	781 ± 44	788 ± 65	841 ± 77	.01	.48

* CA indicates control aged; CY, control young; TA, treated aged; and TY, treated young.
† *P* represents comparison between CA and CY (Mann-Whitney *U* test), $P < .05$.
‡ *P* represents comparison among CY, TA, and TY (1-way ANOVA), $P < .05$.

aging effects. In the case of ANOVA testing, this was followed by the Bonferroni correction. *P* values of less than .05 were considered significant.

Results

Apomorphine-Induced Erection

An apomorphine-induced rhythmic erection was triggered in all the rats at a dose of 100 μg/kg (Table 1). Compared with the results of control young rats, the control aged rats showed various aspects of significant impairment of their erections (Figure 1). There was a prolonged time to the first peak pressure, and the mean maximal ICP was depressed. Correspondingly, the larger AUC was observed in the young control rats. The mean number of erections was not significantly different between the controls. Losartan treatment significantly improved the erectile function of the aged rats. All indices of the apomorphine-induced erection were enhanced by losartan treatment. The mean maximal ICP, the mean time to first response, and the average AUC of the treated aged group were comparable to similar values in the control or losartan-treated young groups.

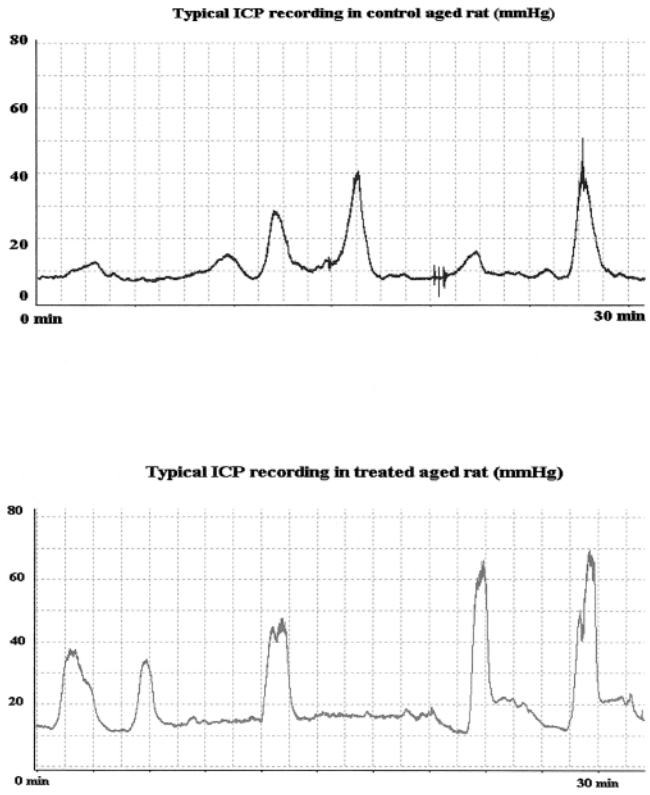

Figure 1. Typical intracavernosal pressure recording in the control aged rats and treated aged rat during apomorphine administration. In contrast to control aged rats, losartan-treated aged rats showed a decreased time to first peak pressure, a higher maximal intracavernosal pressure, and an increased number of erections.

Losartan treatment significantly lowered the mean SAP of both groups of treated rats (Table 2). The ICP/SAP of the aged group, which is related to adequacy of the penile vascular system, was significantly increased by losartan treatment. The rank order of ICP/SAP is as follows; losartan-treated young rats > control young rats; losartan-treated aged rats > control aged rats ($P > .05$, using 1-way ANOVA with Bonferroni correction).

Lipid Peroxidation

Compared to control young rats, the mean level of malondialdehyde in the corporal tissues of control aged rats was significantly increased (12.5 ± 1.2 nmol/gm vs 19.3 ± 1.5 nmol/gm, $P < .001$). This increased rate of lipid peroxidation in aged rats was alleviated by losartan treatment (13.8 ± 0.6 nmol/gm), while there was no significant change observed in the losartan-treated young rats (11.8 ± 1.1 nmol/gm).

Immunoblot

Our results showed the expression of eNOS and TGF-β1 in all the animals. Compared to control young rats, there was approximately a 71% decrease in the estimated eNOS expression in control aged rats (Figure 2). These age-related differences in eNOS were restored after losartan treatment. Like the results of apomorphine-induced erection, the densitogram revealed the improved eNOS expression of the losartan-treated aged rats to the level of the control young rats and the losartan-treated young rats. Similar eNOS expressions were observed between the losartan-treated aged rats and the control young rats.

In contrast, no age or losartan treatment–related differences in TGF-β1 protein expression were observed in the penile tissues of any of the rat groups (Figure 3).

Table 2. *Mean systemic arterial pressure and the ICP/SAP ratio**

	CA	CY	TA	TY	*P*†	*P*‡
Mean SAP	111 ± 3.5	100 ± 4.4	99 ± 3.1	95 ± 3.8	.001	.033
Maximal ICP/mean SAP	0.389	0.641	0.605	0.709	.001	.145

* CA indicates control aged; CY, control young; TA, treated aged; TY, treated young; SAP, systemic arterial pressure; and ICP, intracavernosal pressure.

† *P* represents comparison between CA and CY (Mann-Whitney *U* test), $P < .05$.

‡ *P* represents comparison among CY, TA, and TY (1-way ANOVA), $P < .05$.

Discussion

The results of our study using aged rats indicate that there was an age-related decrease in erectile response to apomorphine, but the decrease was restored by a 4-week administration of AT1 antagonist. Although the role of Ang II in the pathogenesis of age-related erectile dysfunction has not been fully elucidated, our results clearly indicate that Ang II plays a significant role in the impairment of erection that is seen with aging. It is believed that one of the mechanisms of restoration of erectile function is the reduction of vascular resistance. Since Manabe et al (2000) demonstrated that extrapenile vascular resistance primarily controls the arterial blood supply to the penis, and the vascular remodeling caused by Ang II during the aging process may increase the combined inflow resistance from the feeder arteries, arterioles, and the intrapenile vasculature, this subsequently diminishes the blood flow to the penis. Therefore, drugs antagonizing the effect of Ang II are expected to increase the blood flow by inducing vascular relaxation and increased blood flow to the penis. Hale et al (2002) demonstrated a decrease in vascular resistance and an increase in erectile function after only 2 weeks of treatment with enalapril (ACEI) in aged spontaneous hypertensive rats. In addition, the improvement was prominent only in the rats that were receiving enalapril rather than hydralazine (a vasodilator agent). Based on these previous research findings, the restoration of erectile function in normotensive aged rats can be explained as a result of the improved arterial inflow to the penis that resulted from a reduction in vascular resistance by the effective inhibition of Ang II.

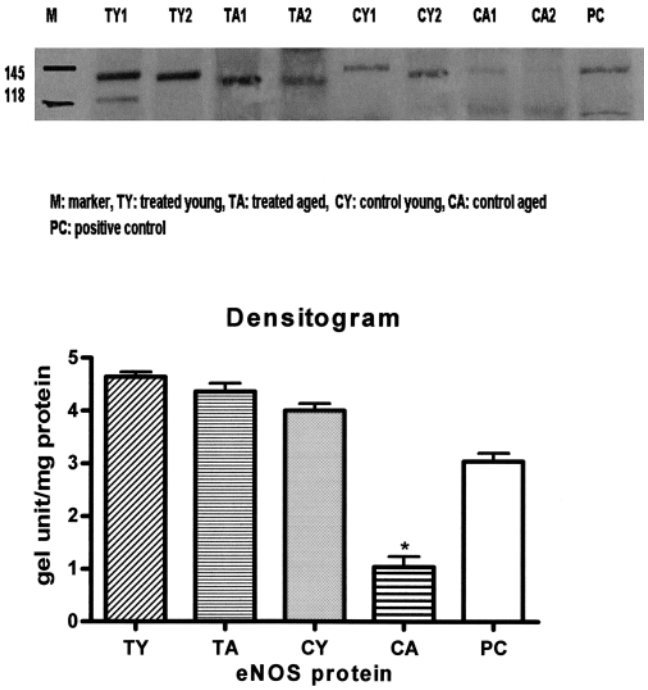

Figure 2. A representative gel picture showing the protein expression of endothelial nitric oxide synthase (eNOS) and the densitogram. A decrease in eNOS was observed in control aged rats. Losartan treatment significantly increased eNOS expression to a level comparable to that observed in young rats. TY indicates treated young; TA, treated aged; CY, control young; CA, control aged; PC, positive control; and M, marker.

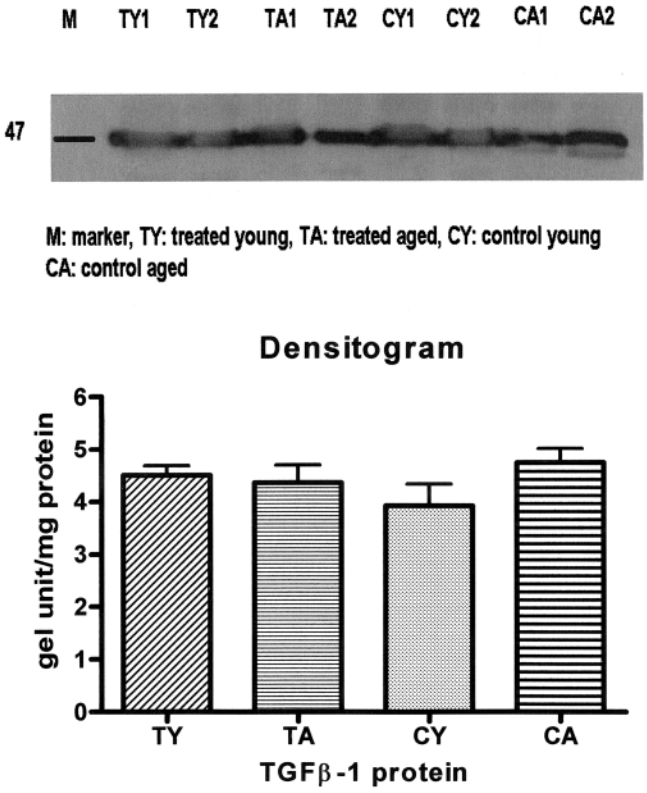

Figure 3. A representative gel picture the showing protein expression of transforming growth factor-β1 and the densitogram. No significant differences were noted in densitograms between groups ($P > .05$). TY indicates treated young; TA, treated aged; CY, control young; and CA, control aged.

Another mechanism of erectile restoration is the alleviation of endothelial dysfunction that impairs penile vasodilation and adequate cavernosal relaxation. Ang II is known to induce endothelial dysfunction via the increased production of reactive oxygen species (ROS) and vasoconstricting eicosanoids, and both of these can counteract the vasodilating and vasoprotective effects of NO (Weber, 2002). The increased generation of superoxide is responsible for NO degradation because it mediates the uncoupling of NO synthase and inactivates NO via peroxynitrite formation. Since age-related endothelial dysfunction is a systemic process, NO (the main effecter of erection) inactivation is thought to occur in the aging penis. In our study, the control aged rats showed an increased production of ROS and decreased eNOS protein expression, and this indicates endothelial dysfunction, while losartan treatment restored these differences to the levels observed for the control young and the losartan-treated young rats.

We have studied the cavernosal expression of two proteins that have been implicated in age-related erectile impairment. Similar to the results of Rajasekaran et al (2002), the eNOS expression of the control aged rat was significantly decreased. The fact that restoration of erection is associated with increased expression of eNOS is also consistent with the results of Champion et al (1999), who demonstrated that the gene transfer of eNOS augmented the erectile response of the aged rat. However, contrary to our expectations, the expression of TGF-β1 is not significantly different among all of the tested rats. This result is contradictory to that of Dahiya et al (1999), who demonstrated an increased mRNA expression of TGF-β1 in senescent (30-month-old) rats. Although the reason for this difference in study results is not completely understood, part of the reason may lie in the age difference between the experimental rat populations. That is, given the possible mechanism of TGF-β1–mediated cavernosal fibrosis that would result from chronic cavernosal ischemia, our aged rats may have been too young to show the increased expression of TGF-β1.

Our findings are similar to those of Hale et al (2002), suggesting that drugs that inhibit Ang II, ACEI, and AT1 antagonists are beneficial to age-related erectile dysfunction; however, AT1 antagonist (such as losartan) has a theoretical benefit over an ACEI (Weber, 2002).

ACEI cannot completely inhibit the action of Ang II to the same degree as the AT1 antagonists. It is well known that ACE inhibitors do not fully prevent the conversion of Ang I to Ang II during chronic therapy, and this is probably because enzymes other than ACE (for example, chymase) may assume a greater role in facilitating this conversion when ACE is blocked. The same phenomenon has also been demonstrated in penile tissue (Iwamoto et al, 2001). This may indicate that long-term ACE inhibition might be ineffective because chymase can also generate Ang II. In contrast to ACE inhibition, AT1 receptor antagonism blocks Ang II completely via the inhibition of the AT1 receptor, and this stops the end results of the receptor activation, such as vasoconstriction and vascular hypertrophy. Furthermore, this may potentially stimulate AT2, which mediates vasodilation and has an antiproliferative effect.

Since the aging rats' erectile function is restored by Ang II blockade, selective AT1 antagonist may be used for the aging male patients who are without cardiovascular disease. Although the current indication of losartan is limited to patients with overt cardiovascular disease, a substudy of the Losartan Intervention for Endpoint Reduction in Hypertension randomized trials has revealed that losartan prevented the incidence of strokes and diabetes even in normotensive patients (Devereux et al, 2003). As subclinical vascular dysfunction appears to play an important role in age-related erectile dysfunction, selective AT1 antagonists may improve vascular health and prevent the progression of clinical erectile dysfunction.

Conclusions

Treatment of normotensive aged rats with AT1 receptor antagonist losartan restored the age-related erectile dysfunction attributable to increased oxidative stress and diminished eNOS expression. Thus, inhibition of the renin-angiotensin system may be an important therapeutic strategy to treat or prevent age-related erectile dysfunction.

References

Akingba AG, Burnett AL. Endothelial nitric oxide synthase protein expression, localization, and activity in the penis of the alloxan-induced diabetic rat. *Mol Urol.* 2001;5:189–197.

Becker AJ, Uckert S, Stief CG, Scheller F, Knapp WH, Hartmann U, Jonas U. Plasma levels of angiotensin II during different penile conditions in the cavernous and systemic blood of healthy men and patients with erectile dysfunction. *Urology.* 2001;58:805–810.

Cartledge JJ, Eardley I, Morrison JF. Nitric oxide–mediated corpus cavernosal smooth muscle relaxation is impaired in ageing and diabetes. *BJU Int.* 2001;87:394–401.

Champion HC, Bivalacqua TJ, Hyman AL, Ignarro LJ, Hellstrom WJ, Kadowitz PJ. Gene transfer of endothelial nitric oxide synthase to the penis augments erectile responses in the aged rat. *Proc Natl Acad Sci U S A.* 1999;116:48–52.

Christ GJ, Stone B, Melman A. Age-dependent alterations in the efficacy of phenylephrine-induced contractions in vascular smooth muscle isolated from the corpus cavernosum of impotent men. *Can J Physiol Pharmacol.* 1991;69:909–913.

Chung WS, Park YY, Kwon SW. The impact of aging on penile hemodynamics in normal responders to pharmacological injection: a Doppler sonographic study. *J Urol.* 1997;157:2129–2131.

Dahiya R, Chui R, Perinchery G, Nakajima K, Oh BR, Lue TF. Differential gene expression of growth factors in young and old rat penile

tissues is associated with erectile dysfunction. *Int J Impot Res.* 1999; 11:201–206.

Devereux RB, Dahlof B, Kjeldsen SE, et al. Effects of losartan or atenolol in hypertensive patients without clinically evident vascular disease: a substudy of the LIFE randomized trial. *Ann Intern Med.* 2003;139: 169–177.

Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB. Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. *J Urol.* 1994;151:54–61.

Hale TM, Okabe H, Bushfield TL, Heaton JP, Adams MA. Recovery of erectile function after brief aggressive antihypertensive therapy. *J Urol.* 2002;168:348–354.

Iwamoto Y, Song K, Takai S, Yamada M, Jin D, Sakaguchi M. Multiple pathways of angiotensin I conversion and their functional role in the canine penile corpus cavernosum. *J Pharmacol Exp Ther.* 2001;298: 43–48.

Kansui Y, Fujii K, Goto K, Abe I, Iida M. Angiotensin II receptor antagonist improves age-related endothelial dysfunction. *J Hypertens.* 2002;20:439–446.

Kifor I, Williams GH, Vickers MA, Sullivan MP, Jodbert P, Dluhy RG. Tissue angiotensin II as a modulator of erectile function. I. Angiotensin peptide content, secretion and effects in the corpus cavernosum. *J Urol.* 1997;157:1920–1925.

Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a ''set up'' for vascular disease. *Circulation.* 2003;107:139–146.

Manabe K, Heaton JP, Morales A, Kumon H, Adams MA. Pre-penile arteries are dominant in the regulation of penile vascular resistance in the rat. *Int J Impot Res.* 2000;12:183–189.

Rajasekaran M, Kasyan A, Jain A, Kim SW, Monga M. Altered growth factor expression in the aging penis: the Brown-Norway rat model. *J Androl.* 2002;23:393–399.

Seftel AD. Erectile dysfunction in the elderly: epidemiology, etiology and approaches to treatment. *J Urol.* 2003;169:1999–2007.

Tarcan T, Azadzoi KM, Siroky MB, Goldstein I, Krane RJ. Age-related erectile and voiding dysfunction: the role of arterial insufficiency. *Br J Urol.* 1998;82:26–33.

Weber MA. The angiotensin II receptor blockers: opportunities across the spectrum of cardiovascular disease. *Rev Cardiovasc Med.* 2002;3: 183–191.

Wespes E. Smooth muscle pathology and erectile dysfunction. *Int J Impot Res.* 2002;14(suppl 1):S17–S21.