Arch. Pharm. Res. Vol.19, No.6, pp. 462-468, 1996

Effects of Age on Angiotensin II Response and Antagonistic Activity of Losartan in Rat Aorta and Liver

Yi-Sook Jung*, Sunghou Lee and Hwa-Sup Shin

Pharmaceutical Screening Center, Korea Research Institute of Chemical Technology (KRICT), Taejon, Korea

(Received July 21, 1996)

The present study was undertaken to investigate the effects of age on angiotensin II (AII) response and antagonistic activity of losartan using aortic rings and liver homogenates from rats ranging in age from 0.7 to 20 months. Whether the endothelium was present or not, the maximum contractile response to AII decreased with age. Removal of the endothelium enhanced AII-induced maximum contraction and these endothelial effects seemed to be due to endothelium-derived relaxing factor (EDRF) in all ages. Equilibrium binding studies demonstrated an age-related decrease in maximum binding (B_{max}) with little change in binding affinity (K_d). In rat aorta, the extent of losartan-induced parallel shifts (K_B) in AII concentration-response curves was not significantly different between ages. In addition, IC_{50} value of losartan in competition binding was not changed with age in rat liver homogenates. These results suggest that the potency of losartan is not altered with age in rat aorta and liver, although AII-induced contractile response and the maximum AII binding decreased significantly with age.

Key Words : Age, Rat aorta, Liver, Losartan, Angiotensin II, Receptor

INTRODUCTION

In both humans and animals, aging is associated with an increased incidence of cardiovascular disorders, e.g., hypertension (Docherty, 1986). Furthermore, age is one of the factors which can influence the responses of tissues to various cardiovascular drugs, including antihypertensive drugs (Fleisch, 1980; Docherty, 1986; Overloop *et al.*, 1993). A number of studies have been reported about the effects of age on antihypertensive drugs, including calcium entry blocking drugs, beta-blockers and angiotensin converting enzyme inhibitors (O'Donnell and Wanstall, 1984; Tsujimoto *et al.*, 1986; Wanstall and O'Donnell, 1988, 1989). However, there is relatively little information about the influence of age on angiotensin II (AII) receptor antagonists, a new class of antihypertensive drug.

The octapeptide AII is the biologically active component of the renin-angiotensin system (Peach, 1977), and plays a key role in the pathogenesis of hypertension. AII receptor antagonists inhibit the renin-angiotensin system by directly blocking AII receptor, and therefore, provide the most specific and effective therapeutic treatment for hypertension (Chiu *et al.*, 1989). Recent studies have shown that AII-induced vasoconstriction changes with age in rat aorta (Wakabayashi *et al.*, 1990; Kuttan and Sim, 1993). These age-related changes in vascular response to AII could be ascribed in part to altered AII receptor function based on recent studies indicating that age-related changes reside at the level of the AII receptor in rat aorta, heart and liver (Ghiani *et al.*, 1988; Kalinyak *et al.*, 1991; Sen and Rajasekaran, 1991; Viswanathan *et al.*, 1991; Lee *et al.*, 1995).

The purpose of the present study was to investigate the influence of age on the pharmacological action of losartan, the prototype of nonpeptide AII receptor antagonists, in the rat. First, using rat aorta and liver, we investigated the effect of age on the contractile response to AII and AII receptor binding properties in more detail. Secondly, we examined the influence of age on the antagonistic activity of losartan in the same assay systems.

MATERIALS AND METHODS

Sprague-Dawley rats used in this study were 0.7 (3 weeks), 1.5, 3, 6, 12 and 20 months of age.

Functional contractile response in isolated rat aorta

The animals were killed by stunning and exanguination. The thoracic aortas were rapidly re-

Correspondence to: Hwa Sup Shin, Pharmaceutical Screening Center, Korea Research Institute of Chemical Technology (KRICT), PO Box. 107, Yousung-Gu, Taejon 305-606, Korea

moved, cleaned of adhering connective tissue, and cut into rings (2-3 mm at length). In some rings the endothelium was removed by gently rubbing the intimal surface with a stainless steel wire. The isolated rings were mounted in 20 ml organ baths by suspending them between two L-shaped stainless steel hooks (Jung *et al.*, 1990). The bath chambers contained the Krebs-Ringer bicarbonate solution (37°C) constantly bubbled with 95% O_2-5% CO_2. The composition of the buffer was (in mM) : NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 10 and $NaHCO_3$ 25 (Wakabayashi *et al.*, 1990). Isometric force was measured by force-displacement transducer (FT03, Grass) and recorded using a Grass 7D polygraph. After mounting, rings were equilibrated for 1 hr with several adjustments of length until the baseline force stabilized at 0.5-1 g, and then the contractile responses to AII (10^{-10}-10^{-5} M) were examined in the presence and absence of endothelium. Only one cumulative concentration-response curve was obtained in each ring preparation to preclude the tachyphylaxis to AII. In separate experiments, the effects of hemoglobin (10^{-5} M) and losartan (10^{-7} M) on AII-induced contractile response were studied. When concentration-response curves for AII were obtained, rings were rinsed several times with Krebs-Ringer bicarbonate solution and allowed to equilibrate for 45-60 min. Following re-equilibration, vascular rings were exposed to KCl solution (120 mM) to obtain the maximum reference response. For all the experiments, the contractile responses to AII were expressed in terms of the percentage of KCl-induced maximum contraction. For the experiments with losartan, data on AII response were further normalized to the percentage of AII (3×10^{-8} M)-induced maximum contraction in vehicle-treated ring from each rat (Emax), and then apparent dissociation constants (K_B) for losartan were calculated using the standard equation of (antagonist)/(dose ratio-1) for comparison of antagonistic potency of losartan among different age groups. All vascular rings were tested to assure the presence or absence of functional endothelium after determining responses to AII. Acetylcholine (10^{-6} M)-induced rapid relaxing response of the ring precontracted with norepinephrine (10^{-7} M) was taken as evidence of a significant amount of functional endothelium, and the disappearance of the relaxing response under the same conditions indicated the absence of endothelium (Gruetter *et al.*, 1988).

Equilibrium binding studies in rat liver homogenates

AII receptors from rat liver homogenates were prepared by minor modifications of a couple of methods (Glossman *et al.*, 1974; Gunther *et al.*, 1980). Livers from rats of various ages were obtained after cervical dislocaton and kept in ice-cold sucrose buffer containing 0.2 M sucrose, 1 mM EDTA and 10 mM Trizma base (pH 7.2). After mincing and rinsing with same buffer, the tissues were disrupted with a Brinkmann Homogenizer (Brinkmann Instruments, Inc.). The homogenate was spun at 3,000×g for 10 min and supernatant was decanted through KimWipes. Collected supernatants were spun at 12,000×g for 13 min. The final supernatant was then centrifuged at 102,000×g for 60 min. The final suspensions in assay buffer containing 0.25% bovine serum albumin (BSA), 5 mM $MgCl_2$, and 50 mM Trizma base (pH 7.2) were divided into 4 ml aliquots and then stored at -70°C. Binding assays were performed in triplicate by incubating aliquots of freshly prepared particulate fraction (0.15-0.20 mg of protein) with various concentrations of [^{3}H] AII with or without inhibitor in 13×100 mm borosilicated glass tubes in a final volume of 0.5 ml. After incubation in a shaking water bath for 60 min at 25°C, the reaction was terminated by the addition of 3 ml of cold washing buffer and the bound radioactivity was separated rapidly through glass fiber filters (GF/C Whatman, presoaked with assay buffer) with a Brandel cell harvester system (Brandel M-12R). The filters were washed with an additional 3 ml of cold washing buffer and trapped radioactivity was measured by a liquid scintillation counter (Packard Tricarb 1500C). All data presented are specific binding, defined as the radioactivity displaceable by 1 μM unlabeled AII added to the assay mixture.

Statistical analysis of the data from functional studies was performed by means of the linear regression and one-way analysis of variance. The level of significance was defined $p<0.05$. Equilibrium binding parameters were obtained using the iterative nonlinear curve fitting program EBDA/LIGAND. All data were expressed as mean±S.E.M.

Drugs used were angiotensin II (Sigma), [^{3}H]AII (5-L-isoleucine, 65 Ci/mmole, DuPont NEN), hemoglobin (Sigma), losartan (synthesized in the laboratory of Dr. S.E. Yoo, KRICT) and lumagel scintillation coctail (Lumac*LSC B.V.). Stock solutions of AII (10^{-3} M) and losartan (10^{-3} M) were prepared in distilled water and dimethyl sulfoxide, respectively, and diluted with appropriate buffer to give a final concentration desired. The mixture of reduced and oxidized forms of hemoglobin supplied by Sigma Chemical Co. was converted to the reduced form as described by Martin *et al* (Martin *et al.*, 1985).

RESULTS

Effect of age on AII-induced contractile response

The contractile response to AII was expressed as

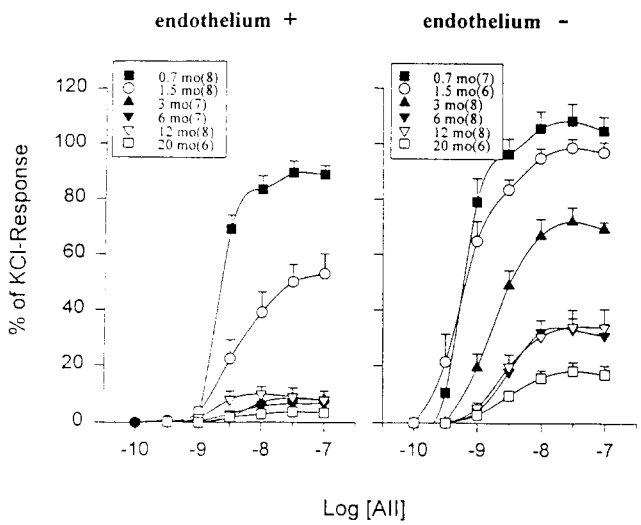

Fig. 1. Dose-response curves for angiotensin II-induced contraction in isolated rat aortic ring with (+) or without (-) endothelium. Data are expressed as percentage of 120 mM KCl-induced contraction in each ring. Each point is the mean and vertical bar indicates the S.E.M. Fig.s in parentheses are numbers of animals, and mo represents month.

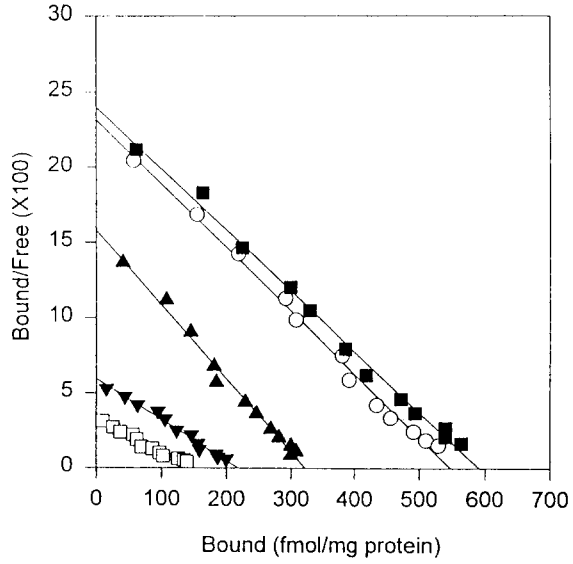

Fig. 2. Scatchard plot analysis of receptor binding in rat liver homogenates. The concentration range of [^{3}H] AII was 0. 1 to 10nM. The liver homogenates were prepared from rats aged 0.7 (■), 1.5 (○), 3 (▲), 6 (▼) and 20 months (□). Data are representative of at least two experiments carried out in triplicate.

the percentage of KCl (120 mM)-induced maximum contraction which was not changed with age (data not shown). Fig. 1 shows the concentration-contractile response to AII in rat aortic rings. Whether the endothelium was present or not, the contractile response to AII decreased with age ($p<0.05$). In rat aorta with endothelium intact, the decrease in maximum contractile response to AII was prominent between the ages of 0.7 to 3 months, with no further significant change noted up to 20 months of age ($p>0.05$). In aorta with endothelium removed, however, the contractile response to AII gradually decreased up to 20 months of age. Removal of the endothelium enhanced AII-induced contraction in rat aorta and the effects of endothelial removal were greatest in aortas from 3-month-old rats.

Equilibrium binding studies

As shown in Fig. 2, a significant age-related decrease in the B_{max} for AII receptor expression was demonstrated in rat liver homogenates : B_{max} (fmol/mg protein) : 0.7 mo 551±5.59, 1.5 mo 548±5.83, 3 mo 298±4.01, 6 mo 202±4.13, 20 mo 140±4.59. The dissociation contants (K_d) were not significantly changed with age (data not shown).

Effect of hemoglobin on AII-induced contraction

In endothelium intact aorta from rats of all ages, an EDRF inhibitor hemoglobin significantly enhanced AII-induced contraction (Fig. 3, $p<0.05$) up to the level of contractile response in endothelium removed aorta. In endothelium removed aorta, hemoglobin had no significant effect on AII-induced contraction.

Antagonistic activity of losartan

As in the the absence of losartan, the maximum contractile responses to AII (expressed as % of KCl-induced maximum contraction) were also decreased with age in the presence of losartan (with endothelium, 0.7 mo 108.71±3.14, 1.5 mo 48.70±8. 83, 3 mo 8.47±2.22, 6 mo 8.52±1.52, 20 mo 5.51± 1.97 ; without endothelium, 0.7 mo 97.75±6.31, 1.5 mo 105.41±3.2, 3 mo 72.34±6.52, 6 mo 36.78±7. 95, 12 mo 18.04±3.92). When data were expressed as the percentage of Emax (3×10^{-8} M AII-induced contraction of vehicle-treated ring preparation in each rat), losartan (10^{-7} M) caused a parallel shift in the concentration-contractile response curves for AII in all ages (Fig. 4). The apparent dissociation constants (K_B) were not significantly different between aortic preparations from young and aged rats (Table I). The specific binding of [^{3}H]AII (3 nM) was inhibited by losartan in a concentration-dependent manner in liver homogenates from young and aged rats (Fig. 5), and IC_{50} values ranged from 0.097 to 0.174 μM (Table I).

DISCUSSION

The influences of age on AII responses and pharmacological action of an AII receptor antagonist losartan were investigated in rat aorta and liver *in vitro*.

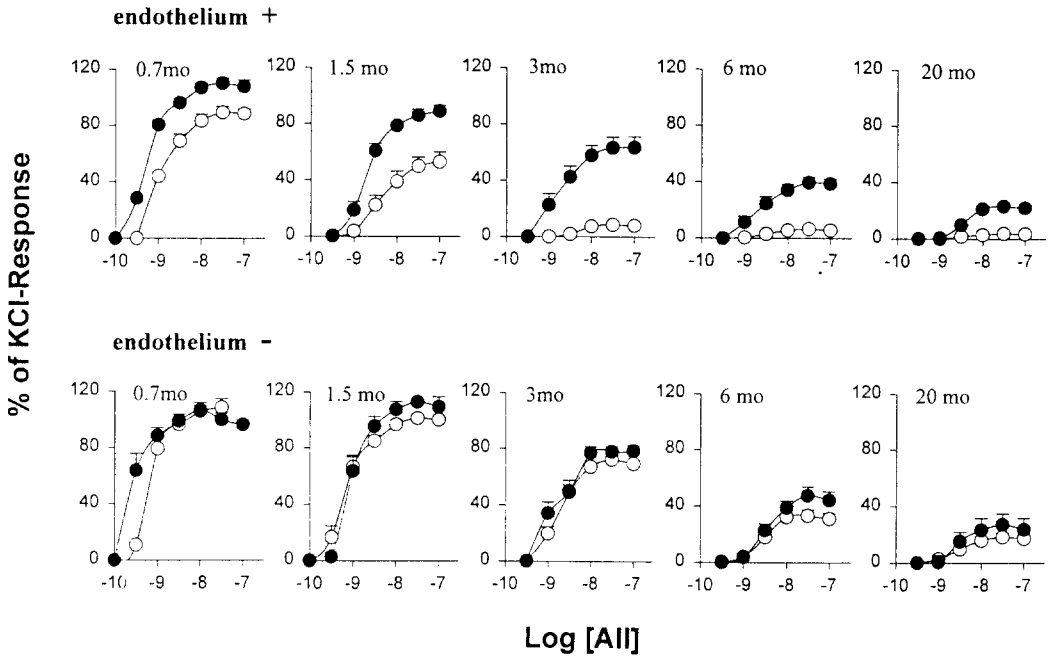

Fig. 3. Effect of hemoglobin on angiotensin II-induced contraction in rings of rat aorta with (+) or without (-) endothelium. Vehicle (saline, open circles) and hemoglobin (10 μM, closed circles) are added 5 min before exposure to AII. Responses are expressed as percentage of the maximum response to 120 mM KCl. Each point represents the mean ± S.E.M. obtained from 6-10 rings (mo represents month). Key: ○, Vehicle; ●, Hemoglobin 10 μM

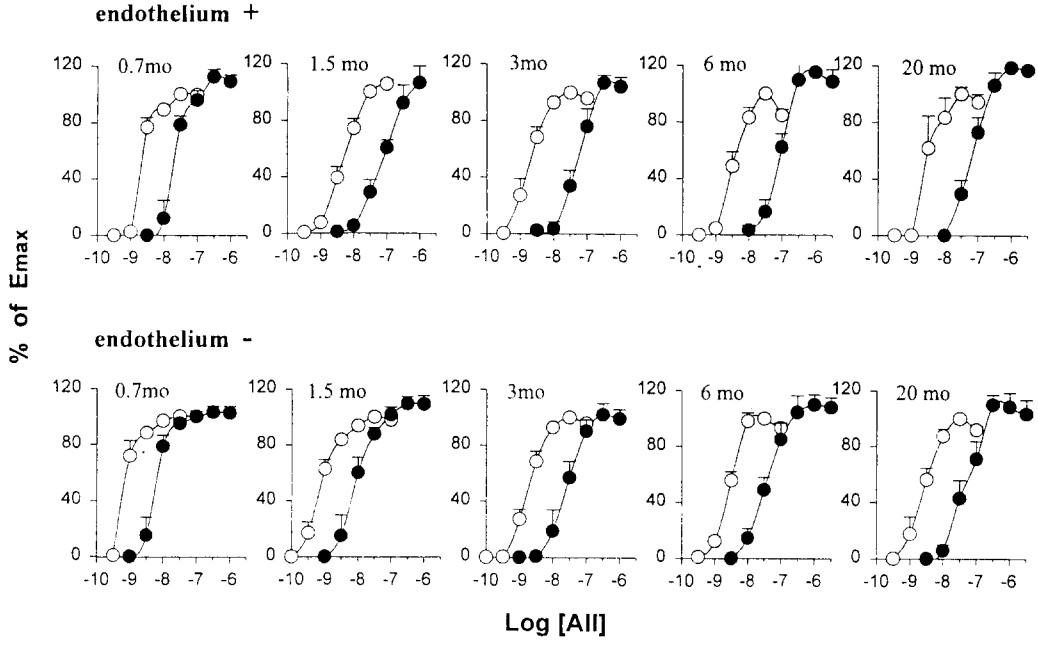

Fig. 4. Dose-response curves for angiotensin II-induced contraction in the presence of losartan in rat aortic rings with (+) or without (-) endothelium. Vehicle (0.1% DMSO, open circles) and losartan (0.1 μM, closed circles) are added 15 min before exposure to AII. Data are expressed as percentage of E_{max} (3×10^{-8} M AII-induced contraction of vehicle-treated control ring in each rat). Each point is the mean of 6-9 rings and vertical bars indicate the S.E.M (mo represents month). Key: ○, Vehicle; ●, Losartan 0.1 μM

The *in vitro* study with isolated tissue has the advantage of excluding multiple *in vivo* factors including reflexes that interfere with the biologic systems of interest. The results from our *in vitro* study have shown that the effect of losartan is not influenced by age, while the AII response itself is

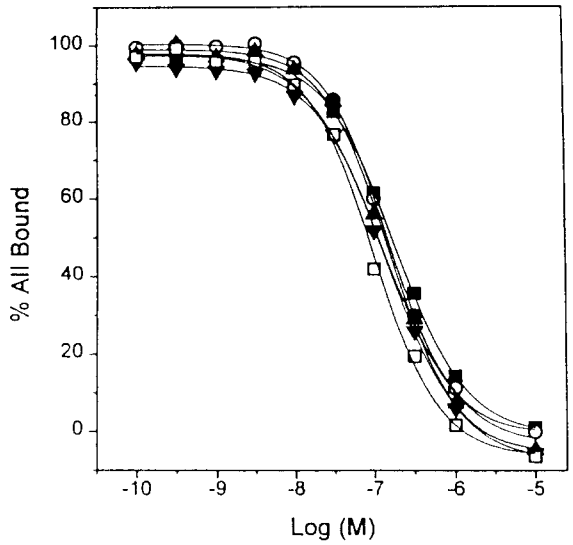

Fig. 5. Competitive binding analysis of losartan. Liver homogenates were prepared from the rats aged 0.7 (■), 1.5 (○), 3 (▲), 6 (▼) and 20 months (□).

changed with age.

Consistent with a recent report (Wakabayashi *et al.*, 1990), the magnitude of the contractile response to AII decreased with age in rat aorta. In order to study the mechanism for the age-related decrease in AII response, we conducted receptor binding study using rat liver homogenates since AII receptor population in rat liver is of the same subtype (AT1) as that found in rat aorta and receptor expression is high enough for binding studies (Pobiner *et al.*, 1985; Bauer *et al.*, 1991). Our results from this study suggest that age-related decreases in the AII response in rat aorta involve the reduction in AII receptor number rather than changes in binding affinity, although we could not directly extrapolate the results from the binding study with liver to the functional study with aorta. This possibility appears to be in line with a recent report that suggests an age-related decrease in the number of AII receptors by studying the maximum binding of AII in immature and adult rat aorta (Viswanathan *et al.*, 1991). It has been also shown that aging (3-20 months) resulted in a gradual decrease in AII mRNA in both rat brain and liver (Kalinyak *et al.*, 1991).

Additional explanations for the mechanism of these age-related changes in AII response may involve age-related alterations in endothelial modulation of vascular responsiveness to AII (Wakabayashi *et al.*, 1990). It has been reported that endothelium-derived relaxing factor (EDRF) is a major mediator in endothelial modulation of AII-induced contractile response in rat aorta and bovine coronary artery (Bullock *et al.*, 1986; Gruetter *et al.*, 1988), although the relationship between age and this inhibitory effect

Table 1. AII antagonistic potencies for losartan shown by functional (K_B values) and competition binding studies (IC_{50} values)

Age (month)	K_B[a] (nM)		IC_{50}[b] (μM)
	Endothelium (+)	Endothelium (-)	
0.7	10.78±2.24	12.40±3.32	0.174
1.5	7.25±1.45	10.30±1.85	0.143
3	9.76±1.91	7.79±1.98	0.151
6	8.24±1.31	9.30±1.73	0.140
20	7.27±1.71	8.74±1.67	0.097

[a]KB value is the apparent dissociation constant calculated from the equation of (antagonist)/(dose ratio-1).
[b]IC_{50} is the inhibitory concentration of losartan producing 50% displacement of the specific binding of labeled AII. The concentration of [^{3}H]AII for competition binding analysis was 3 nM.

of EDRF has not been clear. In this study, a well known EDRF inhibitor hemoglobin (Craver and De Rubertis, 1978; Martin *et al.*, 1985) restored AII contractile responses of endothelium intact aorta up to the level of those in endothelium removed aorta in all ages. These findings indicate that the role of EDRF as a major mediator in counteracting the effect of AII is well preserved in rat aorta irrespective of aging. Further studies should be performed to elucidate the exact mechanism for age-related changes in endothelial modulation of AII response, such as the investigations into the effect of age on the EDRF releasing system.

In many cases, vascular responsiveness to various drugs is either reduced or unchanged by aging. Several studies have been reported that vasodilatory responses to beta-agonists and calcium entry blocking drugs decrease with age in isolated rat blood vessels (O'Donnell and Wanstall, 1984; Wanstall and O'Donnell, 1988, 1989; Overloop *et al.*, 1993). In addition, there are clinical reports that beta-blockers and converting enzyme inhibitors are less effective in elderly hypertensives in lowering blood pressure (Docherty, 1986). A recent report, however, has shown that the potency of losartan in lowering the blood pressure does not decrease with age (Tank *et al.*, 1994), and this result is consistent with our findings in the present study. Losartan (2-n-butyl-4-chloro-5-hydroxymethyl-1-[(2'-(1H-tetrazole-5-yl)biphenyl-4-yl)-methyl] imidazole) is a potent, orally active antihypertensive agent known as the prototype of non-peptide AII receptor antagonists which act as a more selective and newer generation of inhibitors of renin-angiotensin system than angiotensin converting enzyme inhibitors (Wong *et al.*, 1990). It competitively antagonizes the contractile response to AII in rat and rabbit aorta (Chiu *et al.*, 1989), and completely displaces AII binding from its receptors as shown with rat liver homogenates (Bauer *et al.*, 1991). In our

study, the potency of losartan was similar in all age groups in both the functional and the receptor binding experiment. In addition, the presence of functional endothelium did not modify the antagonistic effect of losartan, whatever the age.

In conclusion, the present study suggests that the competitive antagonism and the potency of losartan are not changed with age. The age-related changes in AII-contractile response are likely to reflect the decrease in AII receptor expression and/or the changes in endothelial modulation. Further investigations of the mechanisms for age-related changes in AII responses could lead to insight into the reduced effectiveness of antihypertensive therapy in elderly patients.

ACKNOWLEDGEMENT

The authors would like to acknowledge the efforts of B.C. Oh for his excellent care of animals used in this study. We also acknowledge the kind gift of losartan from Dr. S.E. Yoo.

REFERENCES CITED

Bauer, P. H., Chiu, A. T. and Garrison, J. C., DuP 753 can antagonize the effects of angiotensin II in rat liver. *Mol. Pharmacol.*, 39, 579-585 (1991).

Bullock, G. R., Taylor, S. G. and Weston, A. H., Influence of the vascular endothelium on agonist-induced contractions and relaxations in rat aorta. *Br. J. Pharmac.*, 89, 819-830 (1986).

Chiu, A. T., McCall, D. E., Price, W. A., Wong, P. C., Carini, D. J., Duncia, J. V., Wexler, R. R., Yoo, S. E., Johnson A. L. and Timmermans, P.B.M.W.M., Non-peptide angiotensin II receptor antagonists. VII. Cellular and biochemical pharmacology of DuP 753, an orally active antihypertensive agent. *J. Pharmacol. Exp. Ther.*, 252(2), 711-718 (1989).

Craven, P. A. and De Rubertis, R. F., Restoration of the responsiveness of purified guanylate cyclase to nitrosoguanidine, nitric oxide, and related activators by heme and hemeproteins. *J. Biol. Chem.*, 253, 8433-8443 (1978).

Docherty, J. R., Aging and the cardiovascular system. *J. Auton. Pharmac.*, 6, 77-84 (1986).

Fleisch, J. H., Age-related changes in the sensitivity of blood vessels to drugs. *Pharmac. Ther.*, 8, 477-487 (1980).

Ghiani, P. Uva, B. M., Mandich, A. and Masini, M. A., Angiotensin II vascular receptors I fetal and neonatal rats. *Cell Biochem. Funct.*, 6, 283-287 (1988).

Glossman, H., Baukal, A. J., Catt, K. J., Properties of angiotensin II receptors in the bovine and rat adrenal cortex. *J. Biol. Chem.*, 249, 825-834 (1974).

Gruetter, C. A., Ryan, E. T., Lemke, S. M., Bailly, D. A., Fox, M. K. and Schoepp, D.D., Endothelium-dependent modulation of angiotensin II-induced contraction in blood vessels. *Eur. J. Pharmacol.*, 146, 85-95 (1988).

Gunther, S., Gimbrone, M. A., Jr. and Alexander, R. W., Identification and characterization of the high affinity vascular angiotensin II receptor in rat mesenteric artery. *Circ. Res.*, 47 278-286 (1980).

Jung, Y. S., Kwon, K. I. and Zee, O. P., Differential vasorelaxant effects of KR-30075, a new cyclic AMP-phosphodiesterase inhibitor, on guinea-pig pulmonary, bovine coronary and renal arteries. *Arch. Pharm. Res.*, 13(2), 136-141 (1990).

Kalinyak, J. E., Hoffman, A. R. and Perlamn, A. J., Ontogeny of angiotensinogen mRNA and angiotensin II receptors in rat brain and liver. *J. Endocrinol. Invest.*, 14, 647-653 (1991).

Kuttan, S. C. and Sim, M. K., Actions of norepinephrine and angiotensin II on aortic rings of adult and aged normotensive and hypertensive rats. *J. Cardiovasc. Pharmacol.*, 21, 371-375 (1993).

Lee, S., Jung, Y. S. and Kong, J., Effect of age on angiotensin II receptor binding in rat liver. *Arch. Pharm. Res.*, 18(3), 215-216 (1995).

Martin, W., Villani, G. M., Jothianadan, D. and Furchgott, R. F., Blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation of rabbit aorta by certain ferrous hemoproteins. *J. Pharmacol. Exp. Ther.*, 233(3), 679-685 (1985).

Martin, W., Villani, G. M., Jothianandan, D. and Furchgott, R. F., Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. *J. Pharmacol. Exp. Ther.*, 232, 708-716 (1985).

O'Donnell, S. R. and Wanstall, J. C., Beta-1 and beta-2 adrenoceptor-mediated responses in preparations of pulmonary artery and aorta from young and aged rats. *J. Pharmacol. Exp. Ther.*, 228, 733-738 (1984).

Overloop, B. V., Stoclet, J. C. and Gairard, A., Age, endothelium and aortic reactivity to calcium in wistar rat. *Life Sci.*, 53, 821-831 (1993).

Peach, M. J., Renin-angiotensin system: Biochemistry and mechanisms of action. *Physiol. Rev.*, 57, 313-370 (1977).

Pobiner, B. F., Hewlett, E. L. and Garrison, J. C., Role of Ni in coupling angiotensin receptors to inhibition of adenylate cyclase in hepatocytes. *J. Biol. Chem.*, 260, 16200-16209 (1985).

Sen, I. and Rajasekaran, A. K., Angiotensin II binding protein in adult and neonatal rat heart. *J. Mol. Cell Cardiol.*, 23, 563-572 (1991).

Tank, J. E., Vora, J. P., Houghton, D. C., Anderson, S., Altered renal vascular responses in the aging rat kidney. *Am. J. Physiol.*, 266(6 pt 2), F942-948

(1994).

Tsujimoto, G., Lee, C.-H. and Hoffman, B. B., Age-related decrease in beta adrenergic receptor-mediated vascular smooth muscle relaxation. *J. Pharmacol. Exp. Ther.*, 239(2), 411-415 (1986).

Viswanathan, M., Tsutsumi, K., Correa, F. M. A. and Saavedra, J. M., Changes in expression of angiotensin receptor subtypes in the rat aorta during development. *Biochem. Biophy. Res. Comm.*, 179, 1361-1367 (1991).

Wakabayashi, I., Sakamoto, K., Hatake, K., Yoshimoto, S. and Kurahashi, M., Effect of age on contractile response to angiotensin II in rat aorta. *Life Sci.*, 47, 771-779 (1990).

Wanstall, J. C. and O'Donnell, S. R., Influence of age on calcium entry blocking drugs in rat aorta is spasmogen-dependent. *Eur. J. Pharmacol.*, 159, 241-246 (1989).

Wanstall, J. C. and O'Donnell, S. R., Inhibition of norepinephrine contractions by diltiazem on aorta and pulmonary artery from young and aged rats: influences of alpha-adrenoceptor reserve. *J. Pharmacol. Exp. Ther.*, 245(3), 1016-1020 (1988).

Wong, P. C., Price, W. A., Chiu, A. T., Duncia, J. V., Carini, D. J., Wexler, R. R., Johnson, A. L. and Timmermans, P. B. M. W. M., Nonpeptide angiotensin II receptor antagonists. IX. Antihypertensive activity in rats of DuP 753, an orally active antihypertensive agent. *J. Pharmacol. Exp. Ther.*, 252(2), 726-732 (1990).