Arch. Pharm. Res. Vol. 18, No. 3, pp. 215-216, 1995

Effect of Age on Angiotensin II Receptor Binding in Rat Liver

Sunghou Lee, Yi-Sook Jung and Jaeyang Kong

Pharmaceutical Screening Research Laboratory, Korea Research Institute of Chemical Technology, P.O. Box 107, Yousong, Taejeon 305-606, Korea

(Received December 27, 1994)

Key words: AII receptor, Rat liver, Binding analysis, Angiotensin II, Aging

Angiotensin II (AII), an endogenous peptide which has a potent vasoconstrictive action, plays an important role in the homeostasis of blood pressure and the fluid-electrolyte balance (Peach, 1977). The diverse effects of AII could be mediated by certain types of receptors coupled to specific signal transducers (Douglas, 1987). At least two types of AII receptors (AT_1 and AT_2) have been characterized in specialized tissues, such as adrenal gland, kidney, smooth muscle and liver. The AII receptors show different characteristics in vascular tissues of fetal and neonatal rats (Ghiani *et al.*, 1988), and the soluble AII-binding protein in rat heart reveals an age-related decrease in AII-binding sites (Sen and Rajasekaran, 1991). Some studies have shown that the vascular responses to AII are altered with age in rat kidney and aorta (Tank *et al.*, 1994; Wakabayashi *et al.*, 1990). A recent study demonstrated that the aging from 3 to 20 months caused a gradual decrease in the level of angiotensinogen mRNA in rat liver (Kalinyak *et al.*, 1991), but there is little information about the influence of aging on the AII receptor itself. The present work reports the equilibrium binding properties of [^{3}H] AII to membrane preparations from rat liver aged from 0.7 to 20 months in order to find out the changes in AII receptor during maturation, particularly in the AT_1 receptor, the major mediator of physiological responses to AII.

[^{3}H]AII (5-L-isoleucine, 65 Ci/mmole) was purchased from DuPont NEN (Boston, MA). AII (human) was purchased from Sigma (St. Louis, MO). Lumagel scintillation cocktail was obtained from Lumac*LSC B. V. (Olen, Belgium). All the other chemicals were of the highest purity among commercially available. Male Sprague-Dawley (SD) rats were supplied by Animal Research Lab., KRICT and kept on the standard laboratory chow. The 0.7, 1.5, 3, 6, and 20 month-old SD rats were used as the source of liver. All receptors from rat liver homogenates were prepared by the method of Lee *et al.*, (1995). Briefly, rat livers of various ages were obtained after cervical dislocation 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 the same buffer, the tissues were disrupted with Brinkmann Homogenizer (Brinkmann Instruments, Inc.). The homogenate was spun at 3,000 xg for 10 min and the supernatant was decanted through paper tissue (Kimwipes). Collected supernatant was spun at 12,000 x g for 13 min. The final supernatant was then centrifuged at 102,000 xg for 60 min. The pellet was washed with washing buffer containing 5 mM $MgCl_2$ and 50 mM Trizma base (pH 7.2). All of the above steps were carried out at 4°C. The final pellet was resuspended in assay buffer containing 0.25% bovine serum albumin (BSA), 5 mM $MgCl_2$, and 50 mM Trizma base (pH 7.2).

Binding assays were performed in triplicate by incubating aliquots of freshly prepared particulate fraction (0.15-0.20 mg of protein) with varying concentrations of [^{3}H]AII in 13 x 100 mm borosilicate glass tubes at a final volume of 0.5 ml of assay buffer. 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, prewetted 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). Nonspecific binding was determined in the presence of 1 μM unlabeled AII. Equilibrium binding parameters were obtained using the iterative nonlinear curve fitting program, EBDA-LIGAND (Munson and Rodbard, 1980). The single-site and two-site models for each isotherm were calculated using the differential F value in the LIGAND program.

The assay conditions for all AII receptor preparations were identical (shaking incubation for 60 min at 25°C). The protein concentrations of receptor preparations were adjusted to 1.5 to 2.0 mg/ml for binding experiments. This range of protein concentration was determined by the protein dose analysis (data not shown), and the selected range was

Correspondence to: Sunghou Lee, Pharmaceutical Screening Research Laboratory, Korea Research Institute of Chemical Technology, P. O. Box 107, Yousong, Taejeon 305-606, Korea

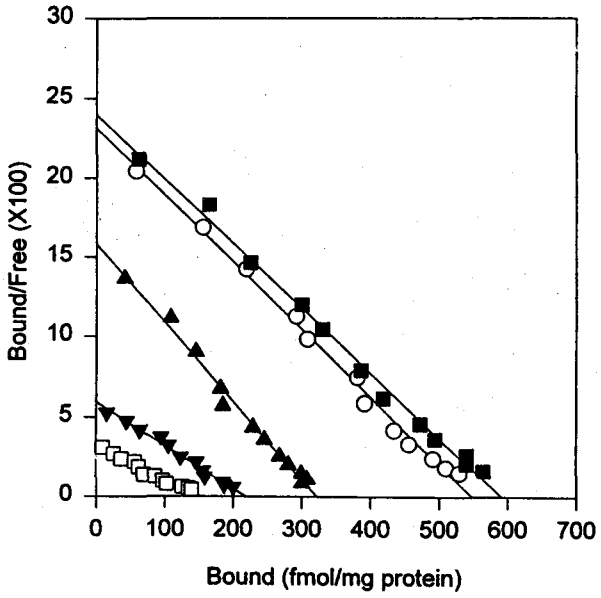

Fig. 1. Scatchard plot from binding of [^{3}H]AII in rat liver homogenates. The concentration range of [^{3}H]AII 0.1 to 10 nM. The liver homogenates were prepared from the rats aged 0.7 (■), 1.5 (○), 3 (▲), 6 (▼) and 20 (□) months.

Table I. Binding parameters of rat liver homogenates

Months of Age	K_d (nM)	B_{max} (fmol/mg protein)	Hill Slopes
0.7	0.90	551	1.04
1.5	0.91	548	1.04
3	0.75	298	1.03
6	1.35	202	0.99
20	1.74	140	1.05

best fitted to the study of [^{3}H]AII binding. In equilibrium binding analysis of rat liver homogenates, the saturability of specific binding was found in all ages. The Scatchard plots of those data showed linear distribution suggesting an interaction of the ligand with a single population of sites (Fig. 1), and it was confirmed by the data analysis through the EBDA-LIGAND program (Table I). The Hill slopes (Hill coefficient, nH) were close to 1 in all ages. Dissociation constant (K_d) values ranged from 0.9 to 1.7 nM in the ages of 0.7 to 20 months, and these values were not significantly different between ages suggesting that binding affinity was not changed during growth. However, an apparent maximum binding (B_{max}) was decreased from 551 to 140 fmol/mg protein with increasing the age from 0.7 to 20 months. Because the rat liver homogenates have very homogeneous population of AT_1 receptor (Lee *et al.*, 1995), these results imply that the number of AT_1 receptor is reduced with aging. The downregulation of [^{3}H]AII binding sites might include the changes in mRNA expression or the increased internalization of AT_1 receptor.

In conclusion, the equilibrium binding studies on AII receptor using rat livers from various months of age demonstrated an age-related decrease in B_{max} with unchanged dissociation constant (K_d). Whether the functional role of AII receptor in rat liver also changes with age remains to be studied.

REFERENCES CITED

Douglas, J. G., Angiotensin receptor subtypes of the kidney cortex. *Am. J. Physiol.*, 253, F1-F7 (1987).

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

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

Lee, S., Lee, B., Shin, H., Kong, J., Rat liver AT_1 receptor binding analysis for drug screening. *J. Appl. Pharmacol.*, 3, 21-27 (1995).

Munson, P. J., Rodbard, D., Ligand: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.*, 107, 220-239 (1980).

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

Sen, I., 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, F942-F948 (1994).

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