BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **218,** 50–53 (1996)
Article No. 0010

Angiotensin Converting Enzyme Inhibitor Normalizes Vascular Natriuretic Peptide Type A Receptor Gene Expression via Bradykinin-Dependent Mechanism in Hypertensive Rats

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Received November 10, 1995

We previously demonstrated that angiotensin converting enzyme (ACE) inhibitor normalizes the up-regulated gene expression of vascular natriuretic peptide type A (NP-A) receptor in hypertensive rats. To elucidate the mechanism, we examined the effect of angiotensin II receptor (AT1) antagonist (TCV-116) and bradykinin receptor (B2) antagonist (Hoe 140) on the NP-A receptor mRNA level in the aorta of genetically hypertensive rats (SHR-SP/Izm) using ribonuclease protection assay. The effect of ACE inhibitor on the NP-A receptor mRNA level was completely abolished by a concomitant administration of Hoe 140, while TCV-116 did not show any significant effect on the NP-A receptor mRNA level. These results suggest that bradykinin plays an important role in the regulation of the vascular NP-A receptor gene expression.

Natriuretic peptide (NP)[2] has been demonstrated to play an important role in the regulation of homeostasis of the body fluid, blood pressure, and vascular structure (for review, see ref. 1, 2, 3, 4). Receptors for NP consist of three subtypes: NP-A receptor with higher affinity to atrial type NP (ANP) and brain type NP (BNP) than C-type NP (CNP), NP-B receptor specific to CNP, and NP-C receptor with similar affinity to all NPs, respectively (5-8). The regulatory mechanism of the receptor however remains unknown.

We have recently demonstrated that gene expression of NP-A receptor is up-regulated in the aorta of hypertensive rats (9) and also that angiotensin converting enzyme (ACE) inhibitor but not calcium channel blockade reverses the up-regulated gene expression of NP-A receptor to the level in normotensive control (10). Since ACE is known to be enzymatically identical to kininase II (11), ACE inhibitor shows its actions by inhibiting both the conversion of angiotensin (Ang) I to Ang II and the breakdown of kinins. In the present study, to elucidate which of the mechanisms is responsible for the effects of ACE inhibitor on the NP-A receptor, we examined the effects of Ang II type 1 receptor (AT1) antagonist and bradykinin B2-receptor antagonist on the NP-A receptor mRNA level in the aorta of hypertensive rats.

MATERIALS AND METHODS

Animals and treatments. Twelve-week-old male stroke-prone spontaneously hypertensive rats (SHR-SP/Izm) were obtained from the Disease Model Cooperative Research Association (Kyoto, Japan) (12). The rats were divided into four groups. The first group was used as the control group. The second group was treated by ACE inhibitor derapril (5mg/kg/day,

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[2] Abbreviations: ACE, angiotensin converting enzyme; NP, natriuretic peptide; AT1, angiotensin II type 1 receptor; SHR-SP/Izm, stroke-prone spontaneously hypertensive rat/Izumo; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CNP, C-type natriuretic peptide; RNase, ribonuclease; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; WKY/Izm, Wistar Kyoto rat/Izumo.

0006-291X/96 $12.00

p.o., Takeda Chemical Industries, Ltd., Osaka, Japan) alone. The third group was treated by derapril (5mg/kg/day, p.o.) and bradykinin B2-receptor antagonist Hoe 140 (75nmol/day, s.c., Hoechst AG, Frankfurt, Germany) (13). The fourth group was treated with AT1 antagonist TCV-116 (1mg/kg/day, p.o., Takeda Chemical Industries, Ltd., Osaka, Japan). The continuous infusion of Hoe 140 was performed using Alzet osmotic minipump (Alza Co., Palo Alto, CA) implanted subcutaneously in the neck of the rats. After 4 weeks of each treatment, rats were killed by decapitation. Aorta was quickly excised, frozen on dry ice, and stored at −80C°.

Systolic blood pressure was measured on the day before death by the tail-cuff method (Manometer-Tachometer, Model KN-210-1, Natume Instruments, Tokyo, Japan) under conscious semi-restrained condition.

Ribonuclease (RNase) protection assay. Total RNA extracts were obtained by the acid guanidinium-thiocyanate-phenol-chloroform method (14). The RNase protection assay for quantitative analysis of the mRNA level was performed according to the method as described previously (9, 10). Antisense cRNA probes of the NP-A receptor and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and synthesized sense RNA standard of NP-A receptor were also generated by the method described previously (9, 10). Twenty μg sample RNA or synthesized sense RNA standard was hybridized with ^{32}P-labeled NP-A receptor probe (1×10^5cpm) overnight at 55°C. For RNA hybridization of the sample, 55C°. For RNA hybridization of the sample, ^{32}P-labeled GAPDH probe (1×10^5cpm) was also added to the hybridization mixture as an internal control. Non-annealing nucleic acids were digested with ribonuclease A and T1, electrophoresed on 5% polyacrylamide gel containing 7M urea, and exposed to X-ray films between two intensifying screens. The integrated optical density of the radioactive bands was determined densitometrically with a computing densitometer (model: ACD- 25DX, ATTO, Tokyo, Japan) and were normalized by that of GAPDH in each sample. NP-A receptor mRNA level in the sample was quantified from the standard curve obtained with known amounts of the synthesized sense RNA and expressed as attomol (amol) mRNA per 20 μg total RNA.

Statistical analysis. Values were expressed as the means ± SEM of multiple experiments. Differences between groups were analyzed by the Student's t test. A P value of less than 0.05 was considered statistically significant.

RESULTS

Systolic blood pressure was 244 ± 2mmHg (n = 4) in the untreated control group, 217 ± 5mmHg in the group treated with derapril (n = 3, $P < 0.05$ vs. control group), and 186 ± 6mmHg in the group treated with TCV-116 (n = 4, $P < 0.01$ vs. control group; $P < 0.05$ vs. group treated with derapril), respectively. Systolic blood pressure in the group treated with derapril and Hoe 140 (211 ± 12mmHg, n = 3) did not show a significant difference from that in the group treated with derapril alone (Fig. 1).

The NP-A receptor mRNA level in the aortic tissue of the untreated SHR-SP/Izm (35.2 ± 0.7 amol/20μg total RNA) was significantly higher than the previously reported level in WKY/Izm (20.2 ± 1.1 amol/20μg total RNA, see Ref. 9). The aortic NP-A receptor mRNA level in the group treated with derapril (23.2 ± 1.3 amol/20μg total RNA) was significant lower than that in the control group ($P < 0.05$). The effect of derapril was, however, completely abolished by the

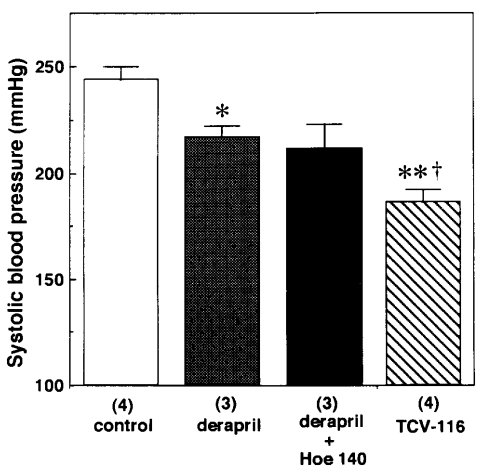

FIG. 1. Effects of derapril, derapril plus Hoe 140, and TCV-116 on the systolic blood pressure in SHR-SP/Izm. Values are the means ± SEM. Number of rats is shown in parentheses. *$P < 0.05$, **$P < 0.01$ vs control; †$P < 0.05$ vs derapril.

concomitant administration of Hoe 140. By contrast, TCV-116 did not show any significant effect on the aortic NP-A receptor mRNA level (Fig. 2).

DISCUSSION

We previously reported that ACE inhibitor, derapril, but not calcium channel blockade, manidipine, normalizes the upregulated gene expression of aortic NP-A receptor in SHR-SP/Izm (10). In the present study, we demonstrated that the effect of derapril is completely reversed by bradykinin B2 receptor antagonist, Hoe 140. In addition, AT1-selective antagonist, TCV-116, did not affect the aortic NP-A receptor gene expression. These findings clearly indicated that the effect of ACE inhibitor on the vascular NP-A receptor gene expression is mediated by bradykinin but not by Ang II.

The lack of effects of AT1 receptor antagonist TCV-116 on the NP-A receptor mRNA level despite its antihypertensive effect more potent than derapril suggests that the high blood pressure is not the major factor modulating the NP-A receptor gene expression. This result agrees with our previous finding with calcium channel blockade (10). This is further supported by the present observation that concomitant administration of bradykinin receptor antagonist Hoe 140 reversed the effect of derapril on the NP-A receptor gene expression without significant change in the blood pressure. It should be noted that the dose of Hoe 140 used in the present study was shown not to affect the blood pressure in normotensive rats (15) and the depressor effect of ACE inhibitor in spontaneously hypertensive rats (16, 17).

Kallikrein-kinin system has also been demonstrated to be involved in the regulation of the body-fluid volume and blood pressure homeostasis (18–20). Although details of the mode of action of the system remain unknown, it has been suggested that local kallikrein-kinin system in the vascular tissue acts in a paracrine fashion (21, 22) to produce vasodilatation via release of nitric oxide and prostaglandins from the endothelium (23). The reversibility of the up-regulated vascular NP-A receptor gene expression by potentiating the kallikrein-kinin system suggests that NP-A receptor gene expression is under the inhibitory regulation by the system. The impairment of this regulatory mechanism may lead to the up-regulation of the NP-A receptor gene expression in SHR-SP/Izm. In support of this hypothesis, kallikrein activity has been reported to be attenuated in the blood vessel of 1K1C hypertensive rats (24) and spontaneously hypertensive rats (25). Further studies are required however to clarify the molecular mechanism of the kinin-dependent

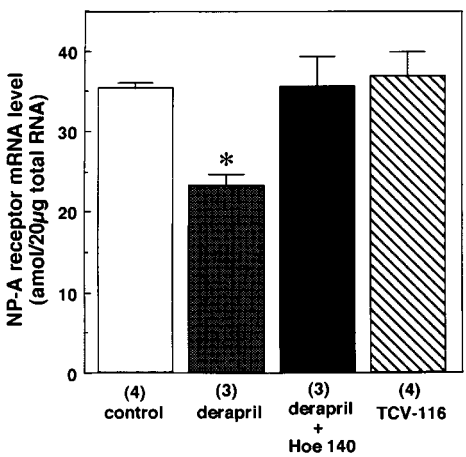

FIG. 2. Effects of derapril, derapril plus Hoe 140, and TCV-116 on the aortic NP-A receptor mRNA level in SHR-SP/Izm determined by RNase protection assay. Values are the means ± SEM. Number of rats is shown in parentheses. *P < 0.05 vs control.

regulation of the NP-A receptor gene expression and the pathophysiological significance of the interaction between the NP system and kallikrein-kinin system.

ACKNOWLEDGMENTS

We thank the Disease Model Cooperative Research Association (Kyoto, Japan) for supplying SHR-SP/Izm, Takeda Chemical Industries, Ltd. (Osaka, Japan) for the supply of derapril and TCV-116, and Hoechst AG (Frankfurt, Germany) for the supply of Hoe 140. This work was supported in part by research grants from the Japanese Ministry of Education, Science, and Culture, the Japanese Ministry of Health and Welfare "Disorders of Adrenal Hormones" Research Committee, Japan, and the Hiroto Yoshioka Memorial Medical Research Award of Tokyo Women's Medical College awarded to M. Naruse, T. Muraki, and H. Demura.

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