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CAPTOPRIL AND HYDRALAZINE SUPPRESS ATRIAL NATRIURETIC PEPTIDE (ANP) GENE EXPRESSION IN THE VENTRICLES OF SPONTANEOUSLY HYPERTENSIVE RAT

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SUMMARY: We investigated the influences of captopril (CAP) and hydralazine (HYD) on the ANP mRNA level in the hypertrophied left ventricle (LV) of spontaneously hypertensive rats (SHR). Male SHRs (16 weeks of age) were given CAP (35 mg/kg/day) or HYD (3.5 mg/kg/day) for two weeks. Both drugs reduced blood pressure by a similar magnitude. Treatment with CAP caused a reduction in the ANP mRNA level in LV by 62%, and a reduction in the weight of the LV. The ANP mRNA level in LV of the HYD-treated rats was also decreased, but only by 31%. HYD did not affect LV hypertrophy. ANP gene expression in LV of SHR might be effectively suppressed by a reduction of blood pressure and also by the concomitant attenuation of hypertrophy. © 1989 Academic Press, Inc.

Atrial natriuretic polypeptide (ANP) is a peptide hormone with potent natriuretic and vasorelaxant activities, synthesized principally in the atria (1, 2). In a non-pathological state, the content of ANP in the cardiac ventricle is negligible (3-5), and its gene expression is markedly lower than that in the atrium (5, 6). However, it has been reported that ANP gene expression increases in the left ventricle of spontaneously hypertensive rats (SHR) (7), and left ventricular hypertrophy is associated with progression of hypertension in SHR (8, 9). These findings

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suggest that hypertrophy and/or hypertension may contribute to augmented expression of the ANP gene in the left ventricle of SHR.

In this study, we tried to discover whether hypertension or hypertrophy was responsible for the augmented ANP gene expression in SHR. Two anti-hypertensive drugs, captopril (CAP) and hydralazine (HYD), which have been reported to produce opposite effects on ventricular hypertrophy, were used. CAP was reported to be effective in the prevention and reversion of ventricular hypertrophy (10-12). HYD does not induce regression of ventricular hypertrophy and may even cause a further potentiation of hypertrophy (8, 13-16). In the present experiments, we administered these drugs to SHRs with established hypertension and hypertrophy, and examined the effects of the drugs on the level of ANP mRNA in the cardiac ventricles.

MATERIALS AND METHODS

Animals

Fifteen male spontaneously hypertensive rats, 12 weeks of age (Charles River Japan Inc.), were used in the present experiments. They were fed ad libitum standard laboratory chow and tap water for a month before the experiments.

At the age of 16 weeks, rats were randomly divided into three groups (5 in each group). Two groups of rats were given CAP (35 mg/kg/day, gift from Sankyo Ltd., Japan) or HYD hydrochloride (3.5 mg/kg/day, gift from Ciba-Geigy Ltd., Japan) in their drinking water for two weeks. Residual rats as control were given tap water to drink. Systolic blood pressure was measured by the tail-cuff method.

Sample Preparation

All the rats used were killed by decapitation. Hearts were rapidly excised and weighed. Right and left auricles were removed. The free walls of the right ventricles were excised and weighed. Left ventricles, including interventricular septa, were weighed, then septa were excised. The top third of the both ventricles and the septum was removed to avoid contamination by atrial tissue. These five parts of the heart (right and left auricle, and apical part of right and left ventricular wall and septum) were frozen with liquid nitrogen, stored separately at -80 °C, and subjected to the extraction of total RNA.

RNA Preparation and Analysis

Total RNA was extracted from individual samples (n=5, for each group) by the methods of Chirgwin *et al.* (17). The amounts of total RNA obtained per gram of each tissue were as follows: left ventricle, 0.52±0.03; right ventricle, 0.73±0.04; septum,

0.58±0.03; left auricle, 0.73±0.04; right auricle, 0.84±0.03 mg. Quantitative measurements of ANP mRNA were performed by Northern blot hybridization analysis as previously reported (18). In short, total RNA samples (3, 6, and 9 μg for ventricle and septum; 0.3, 0.6, and 0.9 μg for auricle) were denatured with 1 M glyoxal and 50% dimethylsulfoxide. To allow for multiple comparison among the three groups, each sample was simultaneously electrophoresed on a 1.5% agarose gel (15 cm × 15 cm, 65 slots) for each part of the heart, and transferred to a nylon membrane (Biodyne A^{TM}; Pall Ultrafine Filtration Corp., Glen Cove, N.Y., USA).

The 368-base pair Hinc II and Stu I fragment derived from cDNA insert of the clone prAFS-1 was labeled with ^{32}P by nick translation and used as a hybridization probe (19). After autoradiography, density of the hybridization image was plotted against the amounts of total RNA applied to each slot, and regression lines were calculated. ANP mRNA levels for individual rats were estimated from the slopes of the regression lines. The relative levels of ANP mRNA were expressed as the ratios to the average value of the slopes for control rats, and compared among the control, CAP- and HYD-treated groups for each part of the heart.

Statistical analysis

The values presented are means±S.E. Student's paired *t*-test was used to assess the statistical significance of the change in blood pressure. For multiple comparisons, a two-way analysis of variance followed by Tukey test was used. P values less than 0.05 were accepted as statistically significant differences.

RESULTS

Fig. 1 shows the effects of CAP and HYD on the blood pressure. Blood pressure of the rats prior to the drug administration (16 weeks of age) was almost the same among the

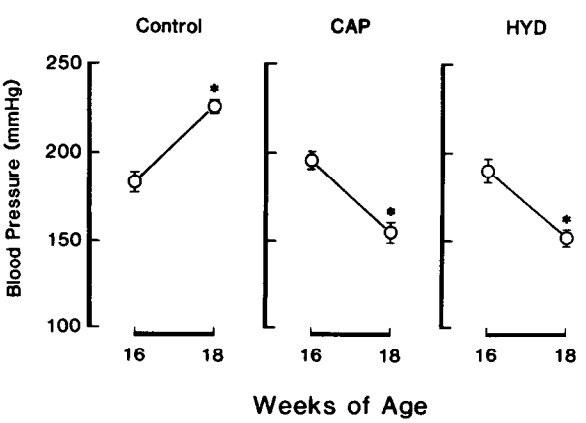

Fig. 1. Effects of treatment with CAP and HYD on the blood pressure of SHR measured by the tail-cuff method. Male SHR (n=5, 16 weeks of age) were given CAP (35 mg/kg/day) or HYD (3.5 mg/kg/day) for two weeks in their drinking water. Asterisks denote significant differences ($p<0.05$) when compared with the values at 16 weeks of age.

three groups. In the control group, blood pressure significantly rose at the age of 18 weeks. In the CAP- and HYD-treated groups, on the other hand, blood pressure significantly reduced by a similar magnitude.

As shown in Table 1, CAP significantly decreased the ratio of left ventricular weight (including septum) to body weight and the ratio of heart weight to body weight, but did not affect the ratio of right ventricular weight to body weight. HYD did not change any of these ratios.

Ventricular ANP mRNA had a single band of ∿950 nucleotides, which was similar to that reported earlier (19). ANP mRNA level in the left ventricle of the control group was approximately 1/45 of that in the auricle, and those in the right ventricle and septum were approximately 1/40 and 2/3, respectively, of that in the left ventricle.

Treatment with CAP significantly reduced the level of ANP mRNA in the right and left ventricle and septum. HYD, on the other hand, only reduced the level of ANP mRNA in the left

Table 1

Effects of captopril and hydralazine on body weight and on the ratios of ventricular weight to body weight

	Control	Captopril	Hydralazine
Body weight (g)	350±6	336±3	336±5
Heart Weight / Body weight (mg/g)	3.66±0.07	3.43±0.04*†	3.62±0.02
(LV+SP) / Body weight (mg/g)	2.78±0.02	2.61±0.03*†	2.74±0.04
RV / Body Weight (mg/g)	0.59±0.01	0.56±0.01	0.60±0.02

LV: Left Ventricle, RV: Right Ventricle, SP: Septum
* P<0.05 vs. Control group; † P<0.05 vs. Hydralazine-treated group; n=5.

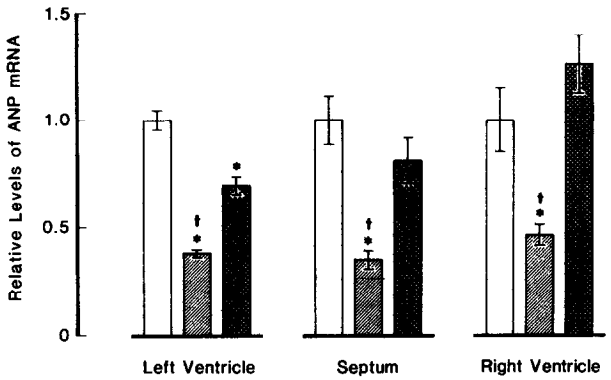

Fig. 2. Relative levels of ANP mRNA in the right and left ventricles and septum of SHRs treated with CAP and HYD. Open, hatched and dotted columns represent control, CAP- and HYD-treated groups, respectively. ANP mRNA levels for each part of the heart are represented as relative values of the slopes of regression lines compared to the average value for the control group designated as 1.0. Asterisks denote significant differences ($p<0.05$) when compared with control. Obelisks denote significant differences ($p<0.05$) when compared with HYD-treated group.

ventricle. The reduction in the left ventricular ANP mRNA level of the CAP-treated group was significantly greater than that of the HYD-treated group (Fig. 2). The level of the right and left auricular ANP mRNA was not affected by treatment with CAP or HYD (data not shown).

DISCUSSION

It has been reported that left ventricular myocardial hypertrophy is demonstrated in hypertensive model rats including SHR (8, 14, 15, 20, 21), and in pressure- or volume-overloaded models (3, 22). Augmented ANP gene expression in the ventricle has been found in hypertrophic models (3, 7, 22, 23). From these findings, we postulated that reduction in blood pressure and/or regression of hypertrophy could prevent the increase of ventricular ANP gene expression in SHR. By using two anti-hypertensive drugs which produce different effects on myocardial hypertrophy, we found the reduction in the ventricular ANP mRNA level in SHR to be caused by reduced blood pressure.

Significant regression of left ventricular hypertrophy was induced in the CAP-treated group, though we gave CAP to SHR for a shorter period than did the studies which showed a drug-induced regression of hypertrophy (8, 10-12, 14, 21). ANP mRNA level in the left ventricle and septum decreased in the CAP-treated group. Left ventricular ANP mRNA level was also decreased in the HYD-treated rats in which blood pressure was reduced to a similar extent as in the CAP-treated rats, and left ventricular hypertrophy was not attenuated. These results suggest that ANP gene expression in the ventricle can be suppressed by drug-induced reduction of blood pressure regardless of the effects of drugs on myocardial hypertrophy.

It has been reported that ANP mRNA level increased in the ventricles of normotensive Wistar-Kyoto rats with biventricular hypertrophy (23). Consequently, degree of hypertrophy may also be a factor which is responsible for the alteration in the ventricular ANP mRNA level. This hypothesis is supported by our findings that CAP-treated rats showed a greater reduction in left ventricular level of ANP mRNA than HYD-treated rats did.

In conclusion, hypertension in SHR may be a factor that enhances left ventricular ANP gene expression; this expression may be potentiated by concomitant progression of ventricular hypertrophy.

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