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# A Novel Angiotensin II-Receptor Antagonist, 606A, Induces Regression of Cardiac Hypertrophy, Augments Endothelium-Dependent Relaxation and Improves Renal Function in Stroke-Prone Spontaneously Hypertensive Rats

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ABSTRACT—It is well-known that cardiac hypertrophy and arterial and renal dysfunction are serious complications of hypertension. Therefore, we investigated the chronic effects of 606A (2-propyl-3-[2′(1*H*-tetrazole-5-yl)biphenyl-4-yl]methyl-5-acetyl-4,5,6,7-tetrahydro imidazo[4,5-*c*]pyridine-4-carboxylic acid disodium salt), a novel $AT_1$-receptor antagonist, on these complications of hypertension in stroke-prone spontaneously hypertensive rats (SHRSP) using Wistar Kyoto rats (WKY) as the control. After 8 weeks treatment from 16 weeks of age with 606A by a subcutaneously implanted osmotic pump, cardiac function, cardiac weight, acetylcholine-induced endothelium-dependent relaxation in the isolated aorta and renal function were estimated. Furthermore, wall thickness of the left ventricle was studied morphologically. We found that 606A (0.3 mg, 1 mg and 3 mg/head/day) dose-dependently lowered blood pressure without any effects on heart rate in SHRSP. Long-term treatments with 606A significantly reduced cardiac weight, left ventricular wall thickness and left ventricular end diastolic pressure, whereas it did not affect cardiac contractility. Endothelium-dependent relaxation of the aorta was recovered, and total protein excretion as well as total protein excretion/creatinine excretion ratio was reduced to the level of WKY by the treatment. These results suggest that 606A not only has a hypotensive effect but also protects cardiac, renal and vascular tissues from complications of hypertension. Thus, 606A could be an useful drug for treatment of hypertension.

*Keywords*: 606A, Angiotensin II-receptor antagonist, Cardiac hypertrophy, Vascular endothelium, Renal function

It is well-recognized that an important clinical target of treatment for hypertension is the improvement of cardiovascular complications such as cardiac hypertrophy, reduced endothelium-dependent relaxation and deteriorated renal function.

Although, increased blood pressure is a major determinant of cardiac hypertrophy in hypertension (1), results of several investigations suggest an involvement of humoral factors in the pathogenesis of hypertrophy in animals. Even in human hypertension, there is some evidence that factors other than pressure overload could be involved; i.e., catecholamines, thyroid hormones, pituitary growth hormone and renin-angiotensin system (RAS) have been proposed as contributing factors (2, 3).

Furthermore, hypertension is also associated with decreased endothelium-dependent relaxations and increased contraction of vascular tissues (4, 5). In the aorta of hypertensive rats, antihypertensive treatments with reserpine, hydrochlorthiazide and hydralazine improve endothelial function (6). The kidney is also an important organ for fluid and electrolytes homeostasis, and its functional abnormality enhances complications in hypertension (7).

Among the available antihypertensive agents, angiotensin converting enzyme (ACE)-inhibitors and angiotensin II-receptor antagonists appear to be potent ones for prevention of cardiac hypertrophy, augmentation of arterial relaxation and improvement of renal impairment (8–12). Recently, losartan has been reported to induce regression of cardiac hypertrophy in spontaneously

hypertensive rats (10), and valsartan augments endothelium-dependent relaxation in the coronary artery of spontaneously hypertensive rats (11). Losartan also well-preserved renal function in patients with essential hypertension (13). Thus, non-peptide angiotensin II $AT_1$-receptor antagonists are considered to be useful drugs for improvement of cardiovascular complications.

The present study was designed to investigate the effects of chronic antihypertensive treatment with a novel, non-peptide angiotensin II $AT_1$-receptor antagonist 606A (2-propyl-3-[2′(1*H*-tetrazole-5-yl)biphenyl-4-yl]methyl-5-acetyl-4,5,6,7-tetrahydro imidazo[4,5-*c*]pyridine-4-carboxylic acid disodium salt) (14), which is more potent than losartan in lowering blood pressure, on cardiac hypertrophy, endothelial function and renal function in stroke-prone spontaneously hypertensive rats (SHRSP).

## MATERIALS AND METHODS

### *Animals*

Male SHRSP (16-week-old) rats, which were originally obtained from K. Okamoto at Kinki University and bred at Tanabe Seiyaku Co., Ltd., were used. Age matched Wistar Kyoto (WKY) (WKY/NCrj) rats were obtained from Charles River Japan (Atsugi). SHRSPs were divided by their body weight, blood pressure and heart rate into 4 groups which were not significantly different from each other (Fig. 1, time at 0): 1) vehicle; 2) 606A, 0.3 mg/head/day; 3) 606A, 1.0 mg/head/day; 4) 606A, 3.0 mg/head/day.

### *Implantation of an osmotic pump*

In the present study, 606A was chronically given subcutaneously by using an osmotic pump (2ML4; Alza Co., Palo Alto, CA, USA) to avoid useless stress to rats. 606A was dissolved in 0.9% NaCl solution at concentrations of 5, 15 and 50 mg/ml. An osmotic pump was filled with one of these solutions to give daily doses of 0.3, 1 and 3 mg/body, respectively. For the vehicle-treated group, the pump was filled with 0.9% NaCl solution. Under ether anesthesia, the osmotic pump was implanted subcutaneously at the back. Because the osmotic pump runs for 4 weeks, the pump was exchanged to a new one after 4 weeks for the 8-week experiment.

### *Measurement of body weight, blood pressure and heart rate*

Body weight, systolic blood pressure and heart rate were measured before and at 1, 2, 4 and 8 weeks after the implantation. Systolic blood pressure and heart rate were measured by tail cuff method (KN-210; Natsume, Tokyo) with preheating to 38℃ to 40℃ for 15 min.

### *Measurement of cardiac function and cardiac weight*

Estimation of cardiac function was carried out 8 weeks after the implantation under thiobutabarbital anesthesia (100 mg/kg, i.p.). Animals were intubated with a tracheal cannula and were ventilated with an artificial respirator (Model-680; Harvard, South Natick, MA, USA). They were placed on a heating table to maintain the body temperature between 36℃ to 38℃ throughout the study. Arterial blood pressure was measured with a Miller catheter pressure transducer (SPR-524; Miller Instruments, Inc., Houston, TX, USA) which was inserted into the right carotid artery and connected to a preamplifier (AP-621G; Nihon Kohden, Tokyo). Heart rate was also measured by a cardiotachometer (AT-601G, Nihon Kohden) triggered by arterial pressure pulses. After stable blood pressure was obtained, the catheter was advanced into the left ventricle to measure left ventricular systolic pressure (LVSP) and left ventricular end diastolic pressure (LVEDP). The first derivative of left ventricular pressure, LVdP/dt, and its corrected value by the left ventricular pressure were consecutively calculated by a differential amplifier (EQ-601G, Nihon Kohden). All measurements were simultaneously recorded on a linearcorder (WR-3310; Graphtech, Tokyo).

After the measurement, the left ventricle (free wall and septum) and the right ventricles (free wall) were dissected free, and weighed. The left ventricle was fixed by 10% buffered formalin and then stained with hematoxylin and eosin. Left ventricular wall thickness at the middle level between the apex and basis cordis was evaluated by measuring the area of the myocardial cross section using an image analyzer (MacSCOPE; Mitani, Fukui).

### *Acetylcholine-induced endothelium-dependent relaxation of aorta*

The thoratic aorta was removed after the estimation of cardiac function. The aorta was dissected free from adherent fat and connective tissue and cut into ring segments of approximately 5-mm width. Vascular rings were suspended in 10-ml organ baths containing Krebs-Henseleit solution that was maintained at 37℃ and aerated with a 5% $CO_2$ / 95% $O_2$ mixture. The composition of Krebs-Henseleit solution was as follows: 118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 25.0 mM $NaHCO_3$, 1.2 mM $KH_2PO_4$, 1.2 mM $MgSO_4$ and 11.4 mM dextrose, pH=7.4. The ring segment was attached to an isometric transducer (UL-10; Minebea, Nagano) connected to a carrier amplifier (AP-601G, Nihon Kohden), and the isometric tension was recorded on a pen-writing recorder (MC6625, Graphtec). A resting tension of 1 g was applied to each segment.

After an equilibration period of at least 90 min, KCl (40 mM) was added to the bath and contractility of the

segment was examined. Then, the medium was repeatedly changed to a fresh one for 90 min. The cumulative concentration-response curve for acetylcholine ($10^{-9}-3\times10^{-5}$ M) was obtained on top of the contraction evoked by prostaglandin $F_{2\alpha}$ (10 $\mu$M). Result was expressed as a percentage of the maximum relaxation due to 100 $\mu$M papaverine, which was added at the end of experiment. The $pD_2$ value, $-\log(EC_{50})$, of each group was determined according to the method of Ariens and van Rossum (15).

*Measurement of renal weight and function*

Animals were housed in individual metabolic cages from 3 days before the end of the study to acclimate them to the environment, and the urine was collected for 24 hr at the end of this period. Urinary sodium and potassium excretions were determined by a colorimetric method, and chloride excretion was determined by a silver electrode method using a standard auto analyzer (Model 710; Hitachi, Tokyo). Urinary creatinine was measured by an enzyme-linked immunosorbent assay (Model 705, Hitachi). Total protein and *N*-acetylglucosamidase excretions were also measured by the auto analyzer (Model 705, Hitachi). After the measurement of cardiac function as described above, blood was collected from the abdominal aorta. Blood urea nitrogen and serum creatinine levels were also measured by the auto analyzer (Model 705, Hitachi). Both the right and left renal weight were measured as wet weight.

*Measurement of plasma concentration of 606A*

After the measurement of cardiac function, blood was collected from the abdominal aorta as described. Plasma was obtained by centrifugation (2800 rpm for 10 min) and stored at −80℃ until determination. Plasma level of 606A was determined at the Analytical Research Laboratory of Tanabe Seiyaku Co., Ltd. (Osaka) by the HPLC-fluorescent method.

*Drugs*

The compound 606A was synthesized at the Lead Optimization Research Laboratory of Tanabe Seiyaku Co., Ltd. (Saitama). Acetylcholine, papaverine and prostaglandin $F_{2\alpha}$ were purchased from Sigma Chemical Company (St. Louis, MO, USA). Other chemicals of the highest grade available were purchased commercially.

*Data analyses*

All data were expressed as means±S.E.M. Statistical analyses were performed by repeated measures of analysis of variance (ANOVA) with Bonferroni correction, and $P<0.05$ was considered to be statistically significant.

## RESULTS

*Chronic effects of 606A on body weight, blood pressure and heart rate*

Figure 1 shows the effects of chronic treatment with 606A on body weight, systolic blood pressure and heart

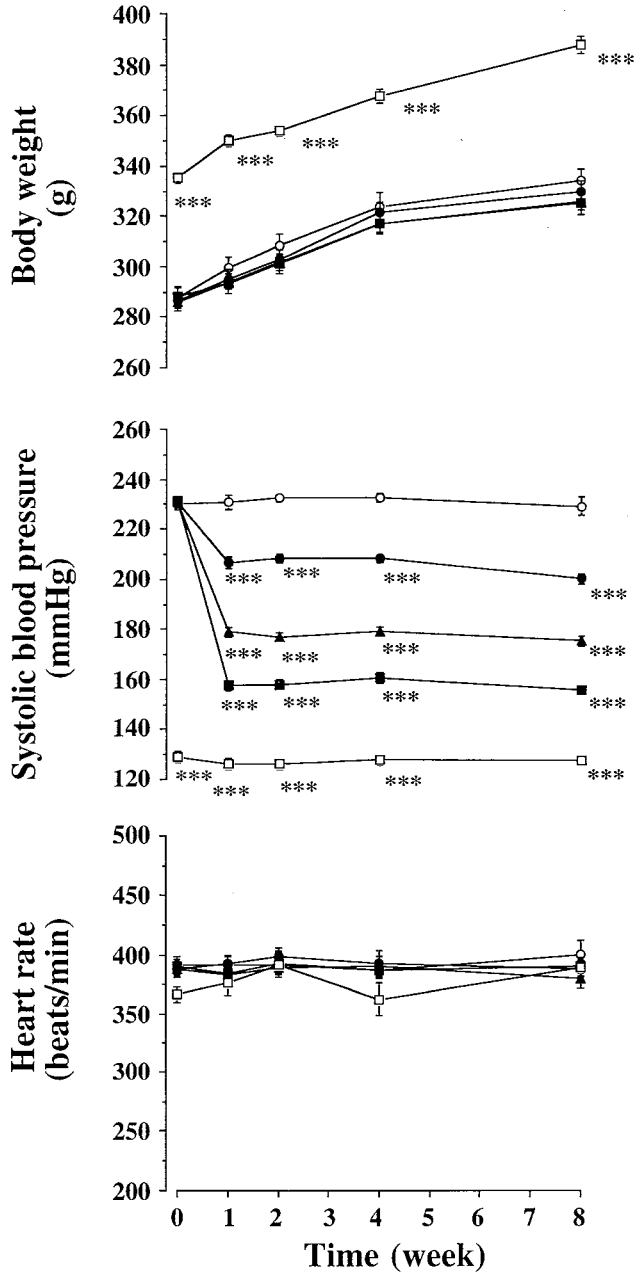


**Fig. 1.** Chronic effects of vehicle (open circle) and 606A at 0.3 mg/head/day (solid circle), 1 mg/head/day (solid triangle) and 3 mg/head/day (solid square) on body weight (upper panel), systolic blood pressure (middle panel) and heart rate (lower panel) in stroke-prone spontaneously hypertensive rats. Wistar Kyoto rats (open square) were used as the normal control. ***$P<0.001$, significantly different from the vehicle group. $n=10-11$.

rate of SHRSP. The same parameters of the normotensive WKY were also plotted as the normal control. SHRSP showed lower body weight, higher blood pressure and comparable heart rate compared to WKY. Although body weights of SHRSP and WKY increased from about 290 g and about 335 g at 16 weeks of age to about 330 g and about 390 g at 24 weeks of age, respectively, systolic blood pressure and heart rate of vehicle-treated SHRSP were maintained at the initial levels of about 230 mmHg and about 400 beats/min, respectively, throughout the experimental period. Chronic treatment with 606A did not affect the natural courses of body weight and heart rate, but significantly reduced the blood pressure in a dose-dependent manner. The maximum hypotensive response to 606A was observed even a week after the start of experiment. The effect was stable throughout the study.

*Chronic effects of 606A on cardiac function and cardiac weight*

The left and right ventricular weight, which are normalized by the body weight (LV/BW and RV/BW, respectively), are shown in Fig. 2. Both LV/BW and RV/BW in the vehicle-treated SHRSP were significantly ($P<0.001$) larger than those in WKY. Chronic treatment with 606A significantly reduced LV/BW and RV/BW in a dose-dependent manner. LV/BW in the highest dose group was comparable to that in WKY.

LVSP was lowered by 606A in a dose-dependent manner and was in proportion to their blood pressure. LVEDP in vehicle-treated SHRSP was significantly ($P<0.001$) higher than that in WKY. The elevated LVEDP was lowered by 606A in a dose-dependent manner. Although $LVdP/dt_{max}$ in vehicle-treated SHRSP was significantly larger than that in WKY and was reduced by 606A treatment, there were no differences among groups in $LVdP/dt/P_{max}$, which is the corrected value of $LVdP/dt_{max}$ by the left ventricular pressure (Table 1).

Calculated cross sectional area of the left ventricular wall in the vehicle-treated group of SHRSP was $74.7\pm2.2$ $mm^2$, whereas that of WKY was $67.0\pm2.4$ $mm^2$. The area was reduced by 606A to $68.3\pm1.3$ $mm^2$ at a dose of 1 mg/head/day and to $67.5\pm0.4$ $mm^2$ at a dose of 3 mg/head/day (Fig. 3).

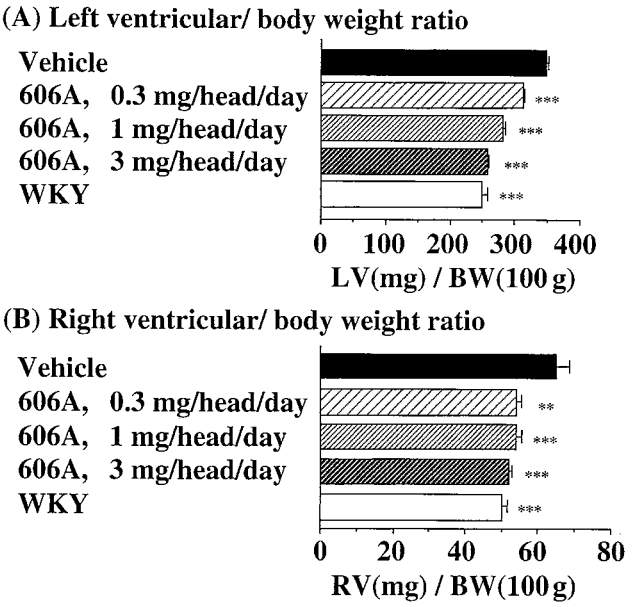


**Fig. 2.** Chronic effects of vehicle and 606A at 0.3, 1 and 3 mg/head/day on left ventricular weight (mg)/body weight (100 g) ratio (A) and right ventricular weight (mg)/body weight (100 g) ratio (B) in stroke-prone spontaneously hypertensive rats. Wistar Kyoto (WKY) rats were used as the normal control. **P<0.01 and ***P<0.001, significantly different from the vehicle group. n=10–11.

*Effects of 606A on acetylcholine-induced endothelium-dependent relaxation*

Acetylcholine-induced endothelium-dependent relaxation in the aorta of SHRSP and WKY is shown in Fig. 4, and the maximum relaxation and $pD_2$ value due to acetylcholine are described in Table 2. The maximum relaxation (g) due to 100 $\mu$M papaverine was

**Table 1.** Effects of 606A on mean blood pressure, heart rate and cardiac function in anesthetized stroke-prone spontaneously hypertensive rats

| Group | MBP (mmHg) | HR (beats/min) | LVSP (mmHg) | LVEDP (mmHg) | $LVdP/dt_{max}$ (mmHg/sec) | $LVdP/dt/P_{max}$ (1/sec) |
|---|---|---|---|---|---|---|
| Vehicle | 237.0±3.9 | 320.0± 4.8 | 260.5± 8.8 | 10.6±1.0 | 14034.2±415.2 | 375.7±27.3 |
| 606A, 0.3 mg/head/day | 213.6±9.0* | 301.2±14.0 | 237.1±10.4 | 8.5±0.8 | 12454.3±573.8 | 373.3±25.0 |
| 606A, 1 mg/head/day | 194.6±5.3*** | 311.5±13.6 | 219.6± 5.9** | 6.9±0.8** | 12073.7±352.3* | 378.1±26.3 |
| 606A, 3 mg/head/day | 170.8±4.5*** | 316.5±17.4 | 190.3± 4.9*** | 5.6±0.9*** | 10998.8±435.1*** | 389.9±24.4 |
| WKY | 130.1±6.6*** | 311.8±17.0 | 146.1± 5.3*** | 4.2±0.6*** | 9035.3±438.7*** | 368.0±23.4 |

Wistar Kyoto (WKY) rats were used as the normal control. MBP: mean blood pressure, HR: heart rate, LVSP: left ventricular systolic pressure, LVEDP: left ventricular end-diastolic pressure. *P<0.05, **P<0.01 and ***P<0.001, significantly different from the vehicle group. n=10–11.

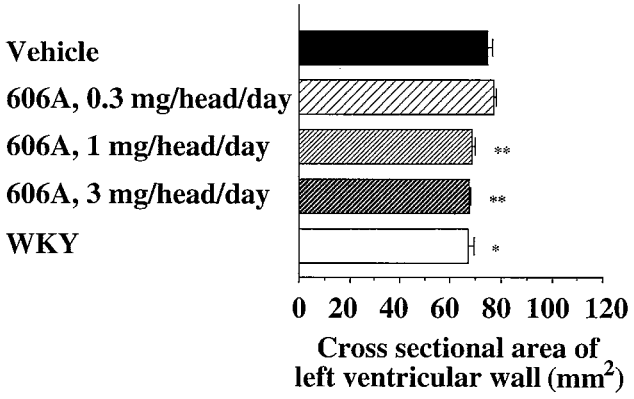


**Fig. 3.** Effect of 606A on cardiac ventricular hypertrophy in stroke-prone spontaneously hypertensive rats. Wistar Kyoto (WKY) rats were used as the normal control. *P<0.05 and **P<0.01, significantly different from the vehicle group. n=10-11.

**Table 2.** Effects of 606A and $pD_2$ and the maximum response to acetylcholine in isolated stroke-prone spontaneously hypertensive rat aorta

| Group | $pD_2$ | Maximal response |
|---|---|---|
| Vehicle | 7.28±0.08 | 37.32±2.21 |
| 606A, 0.3 mg/head/day | 7.43±0.08 | 47.36±1.84* |
| 606A, 1 mg/head/day | 7.51±0.05* | 47.79±3.50* |
| 606A, 3 mg/head/day | 7.64±0.11** | 49.02±4.71* |
| WKY | 7.50±0.08* | 48.11±3.54* |

Wistar Kyoto (WKY) rats were used as the normal control. *P<0.05, **P<0.01, significantly different from the vehicle group. n=6-8.

not different among the groups: 1.30±0.25 (vehicle), 1.34±0.11 (606A, 0.3 mg/head/day), 1.33±0.13 (606A, 1 mg/head/day), 1.29±0.23 (606A, 3 mg/head/day), 1.32±0.10 (WKY). The maximum value of acetylcholine-induced endothelium-dependent relaxation in the aorta of vehicle-treated SHRSP was significantly smaller than that of WKY, whereas threshold concentration of acetylcholine to induce relaxation was same between these groups. Their $pD_2$ values were also significantly different from each other. Chronic treatment with 606A enhanced acetylcholine-induced relaxation to the level of WKY, although the dose-dependency was weak. The aorta obtained from 606A-treated SHRSP demonstrated com-

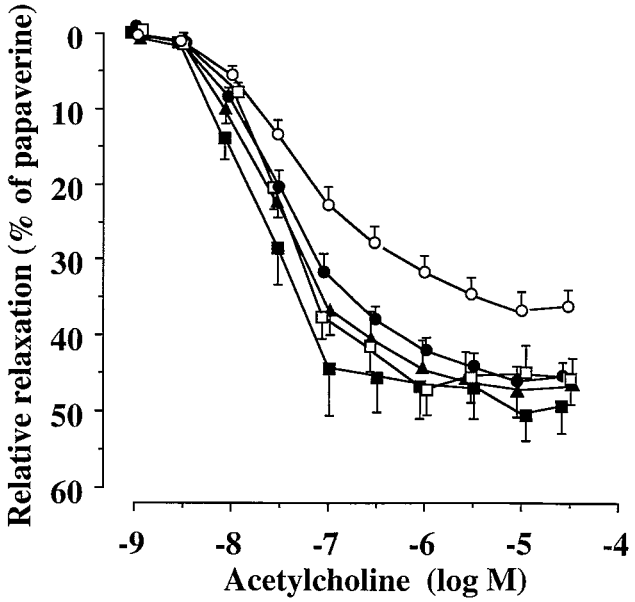


**Fig. 4.** Effects of vehicle (open circle) and 606A at 0.3 mg/head/day (solid circle), 1 mg/head/day (solid triangle) and 3 mg/head/day (solid square) on acetylcholine-induced endothelium-dependent relaxation in the aorta of stroke-prone spontaneously hypertensive rats. Wistar Kyoto rats (open square) were used as the normal control. n=6-8.

**Table 3.** Effects of 606A on renal weight and urine parameters in stroke-prone spontaneously hypertensive rats

| Group | RW (mg/100 g BW) | UV (mg/100 g/day) | UNaV (μEq/day) | UKV (μEq/day) | UClV (μEq/day) | UCreV (mg/day) | TP (mg/day) | TP/UCreV ratio | UNAGV (U/day) | GFR (ml/hr) | BUN (mg/dl) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Vehicle | 425.1 ±14.1 | 3.2 ±0.2 | 1078 ±44 | 3064 ±153 | 1414 ±64 | 7.32 ±0.31 | 24.6 ±1.1 | 3.41 ±0.21 | 0.31 ±0.01 | 82.2 ±5.7 | 28.1 ±4.6 |
| 606A, 0.3 mg/head/day | 447.0 ± 9.9 | 2.8 ±0.2 | 913 ±71 | 3020 ±210 | 1312 ±83 | 7.09 ±0.36 | 19.6 ±1.3* | 2.77 ±0.11* | 0.30 ±0.02 | 82.5 ±6.4 | 27.7 ±5.7 |
| 606A, 1 mg/head/day | 434.5 ±17.6 | 2.7 ±0.2 | 1076 ±34 | 3316 ± 71 | 1396 ±43 | 7.10 ±0.18 | 17.3 ±0.7*** | 2.44 ±0.09*** | 0.30 ±0.02 | 80.2 ±5.2 | 27.8 ±4.7 |
| 606A, 3 mg/head/day | 428.4 ± 8.9 | 2.3 ±0.2** | 1011 ±31 | 3134 ±123 | 1246 ±76 | 7.71 ±0.51 | 17.1 ±1.9*** | 2.18 ±0.11*** | 0.27 ±0.02 | 85.0 ±9.2 | 35.2 ±6.0 |
| WKY | 329.6 ±12.9* | 4.7 ±0.3 | 1168 ±51 | 3333 ± 70 | 1469 ±32 | 10.29 ±0.24*** | 11.7 ±0.7*** | 1.14 ±0.07*** | 0.32 ±0.03 | 104.7 ±7.0* | 29.3 ±5.8 |

Wistar Kyoto (WKY) rats were used as the normal control. RW: renal weight, UV: urine volume, UNaV: urinary sodium excretion, UKV: urinary potassium excretion, UClV: urinary chloride excretion, UCreV: urinary creatinine excretion, TP: total protein excretion, UNAGV: urinary *N*-acetylglucosamidase excretion, GFR: glomerular filtration rate, BUN: blood urea nitrogen. *P<0.05, **P<0.01 and ***P<0.001, significantly different from the vehicle group. n=10-11.

parable maximum relaxation and $pD_2$ to those of WKY.

*Effects of 606A on renal weight and function*

Table 3 represents the results on renal weight and function. Renal weights, which were normalized by body weight, were similar to each other in all SHRSP groups, although that of WKY was significantly smaller than those of SHRSP. Urine volume of vehicle-treated SHRSP was less than that of WKY, but the difference was not statistically significant. The urine volume of 606A 3 mg/head/day-treated SHRSP was significantly reduced, but electrolytes excretion were not affected by the treatment with 606A. Total protein excretion of SHRSP was significantly larger than that of WKY even if the excretion was corrected by creatinine excretion. Although the difference was not apparent, 606A reduced the protein excretion of SHRSP significantly.

*Plasma concentration of 606A*

Plasma concentrations of 606A (ng/ml) depended on the doses we used, and were as follows (group of 606A): $64.6 \pm 10.0$ (0.3 mg/head/day), $166.5 \pm 32.0$ (1 mg/head/day), $352.1 \pm 51.7$ (3 mg/head/day).

## DISCUSSION

In the present study, we demonstrated the effects of 606A, a novel non-peptide angiotensin II $AT_1$-selective receptor antagonist (14), on cardiac hypertrophy, endothelium-dependent relaxation of the aorta and renal function in SHRSP.

It was clear that the osmotic pump ran well during the experiment, because we observed not only a dose-dependent hypotensive effect of 606A but also a dose-dependent plasma level of 606A.

RAS plays a major role in regulation of blood pressure and morphological alterations of cardiovascular organs via angiotensin II (16–18). Increasing evidence suggests the existence of local RAS with paracrine and autocrine functions in addition to the systemic endocrine system. Cardiac hypertrophy observed in hypertensive animal models or in humans seems to depend not only on blood pressure but also on local RAS (19). It was already reported that angiotensin II $AT_1$ blockade was more effective for reducing cardiac hypertrophy than calcium channel blockade (20). Bruckschlegel et al. also reported that an ACE-inhibitor, ramipril, or an angiotensin II $AT_1$-receptor antagonist, losartan, could prevent cardiac hypertrophy, whereas hydralazine, which exhibited a hypotensive effect equipotent to those of ramipril and losartan, did not affect the cardiac hypertrophy (21).

In the present study, the left and right ventricular weight were significantly increased in SHRSP, and increased LV/BW and RV/BW were markedly reduced by chronic treatment with 606A in a dose-dependent manner. These effects of 606A are consistent with a previous report (10). It was also reported that losartan decreased mRNA levels for myocardial phenotype- and fibrosis-related genes; i.e., skeletal alpha-actin, atrial natriuretic peptide, transforming growth factor-beta-1 and collagen types I, III, IV, and increased adult phenotype of contractile protein (alpha-MHC) mRNA in the left ventricle of SHRSP (20). Therefore, it is possible that 606A influences these genes like losartan.

Our study further showed that LVEDP in SHRSP was significantly higher than that in WKY, although the contractile function of SHRSP was normal as shown by comparable $LVdp/dt/P_{max}$ in SHRSP to that in WKY. In general, impairment of relaxation precedes impaired contractile function in cardiac hypertrophy (22). A similar phenomenon was observed in our SHRSP. Elevated LVEDP causes stress to the left ventricular muscle and should deteriorate the contractile function in the future. It is demonstrated in the present study that 606A improved this risk factor in a dose-dependent manner. Therefore, angiotensin II receptor antagonist should be one of the drugs that reduce the risk of a cardiac event.

Furthermore, chronic treatment with 606A improved the endothelium-dependent relaxation evoked by acetylcholine to the level of WKY, although the dose-dependency of 606A was slight. As described in the result, papaverine-induced relaxation was comparable among all groups including WKY. Therefore, it was considered that chronic treatment with 606A did not affect papaverine-induced relaxation. On the contrary, endothelium-dependent relaxation of blood vessels has been reported to be impaired in hypertensive rats (23, 24). The present study also demonstrated that the endothelium-dependent relaxation was markedly impaired in the preparation from vehicle-treated SHRSP when compared with that from WKY. Because impairment of endothelium-dependent relaxation is reported to be proportional to blood pressure and to duration of exposure to hypertension (25), the prevention of impairment in endothelium-dependent relaxation by 606A may be explained by its hypotensive action. However, Clozel et al. (26) reported that improvement of endothelial function was observed in rats treated with ACE-inhibitors, but not in those treated with hydralazine. Thus, the inhibition of RAS may be particularly effective for preventing changes in endothelium in spontaneously hypertensive rats.

Blockade of angiotensin II $AT_1$ receptor is reported to increase glomerular filtration rate (GFR) (27, 28). In our study, however, GFR was not altered by 606A as previously reported in essential hypertension using losartan (13, 29). Such a discrepancy may be due to experimental

and animal conditions. There were no apparent differences in sodium, potassium and chloride excretions between the 606A-treated group and vehicle-treated group, although urine volume was reduced by 606A at the dose of 3 mg/head/day. Total protein excretion was reduced by 606A in a dose-dependent manner. Total protein excretion/creatinine excretion ratio was also reduced by 606A in a dose-dependent manner. Thus, it is considered that 606A protects the glomerular barrier function, whereas GFR is not changed by 606A. Our result is consistent to the report that angiotensin II antagonism caused amelioration of membrane-sieving function (30). The development of glomerulosclerosis might be inhibited by 606A.

In conclusion, we have demonstrated that 606A not only decreases blood pressure but also causes regression of cardiac hypertrophy, augments endothelium-dependent relaxation and inhibits protein excretion in SHRSP. These results suggest 606A can be an useful drug for cardiovascular complications.

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