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Control of vascular changes by renin–angiotensin–aldosterone system in salt-sensitive hypertension

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Abstract

The purpose of this study was to investigate the role of the renin–angiotensin–aldosterone system in hypertension development and cardiovascular structural changes in a salt-sensitive hypertensive model induced by capsaicin (CAP). Newborn male Wistar rats were injected with either capsaicin or vehicle. After weaning at 3 weeks, these rats were divided into the following six treatment groups: capsaicin plus high-salt diet (CAP+HS), control plus high-salt diet (CON+HS), control plus normal salt diet (CON+NS), capsaicin plus high-salt diet and telmisartan (CAP+HS+T, 10 mg/kg/day), capsaicin plus high-salt diet and perindopril (CAP+HS+P, 2 mg/kg/day), and capsaicin plus high-salt diet and spironolactone (CAP+HS+S, 80 mg/kg/day). Treatment with different salt diets and drugs was initiated at 3 weeks of age and lasted 18 weeks. We found that beginning at the second week after the initiation of the treatment, blood pressure became significantly higher in CAP+HS than in other groups, accompanied by the development of cardiac hypertrophy. Treatment with telmisartan, perindopril or spironolactone prevented the development of hypertension in the CAP+HS group. Cardiac hypertrophy was prevented in the perindopril treatment group. The medial thickness, media-to-lumen ratio and cross-sectional area of the thoracic, renal and mesenteric arteries were significantly increased in CAP+HS than in other groups. Lumen diameter was similar in all the vessels among all the groups. Treatment with telmisartan, perindopril or spironolactone prevented the development of vascular remodeling, as found in the CAP+HS group. Beginning at 8 weeks after the initiation of the salt diet treatment (11 weeks of age), blood pressure also became higher in CON+HS than in CON+NS, but lower than CAP+HS. Structural changes of vessels were also present in CON+HS, but the degree of change was less when compared with CAP+HS. We conclude that neonatal treatment with capsaicin plus a high-salt diet, and a high-salt diet alone both induced hypertension development in normal Wistar rats, which are associated with cardiovascular remodeling. The renin–angiotensin–aldosterone system is involved in this salt-sensitive model because treatment that interfered with this system also prevented the development of hypertension and vascular remodeling.

Keywords: Vascular; Remodeling; Capsaicin; Hypertension; Salt; Heart

1. Introduction

Excessive salt intake is an important determinant of human essential hypertension (Jones, 2004). The neonatal destruction of capsaicin (CAP)-sensitive sensory nerves and a high-salt diet can cause an increase in blood pressure and a decrease in natriuretic response in normal rats (Wang et al., 1998). We have recently shown that structural changes of the blood vessels are involved in this new animal model of hypertension (Zeng et al., 2004). We have also found that chronic high-salt diet also induced the development of hypertension in normotensive rats, and this was also associated with the development of cardiovascular structural changes (Zeng et al., 2004).

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The studies by Wang et al. showed that concomitant treatment with angiotensin II receptor antagonists (Huang and Wang, 2001a; Wang and Li, 1999) or aldosterone receptor antagonist (Huang and Wang, 2001b) prevented the development of hypertension in this sensory deprivation hypertension model, indicating the importance of the renin–angiotensin–aldosterone system in this model. However, in these studies, treatment with various receptor antagonists lasted only 2–3 weeks. The effects of a longer treatment on blood pressure regulation are not known. The effects of blocking the renin–angiotensin–aldosterone system on cardiovascular changes in this model are also unknown. The purpose of the study was to investigate the role of the renin–angiotensin–aldosterone system on the changes in arterial structure and hypertension development in this salt-sensitive hypertensive model induced by capsaicin, through the use of three types of antihypertensive drugs: perindopril (an angiotensin converting enzyme inhibitor), telmisartan (an angiotensin AT_1 receptor antagonist) and spironolactone (an aldosterone receptor antagonist).

2. Materials and methods

2.1. Animals

Wistar rats were obtained from the colonies maintained at the Laboratory Animal Center of Guangzhou Medical College. These colonies originated from the Laboratory Animal Center, National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) in 1999. Newborn male Wistar rats were injected with 50 mg/kg capsaicin (CAP) subcutaneously on the first and second days of life. Control rats were injected with an equal volume of the vehicle solution (5% ethanol and 5% Tween 80 in saline). After weaning at 3 weeks, blood pressure and body weight were measured. Male rats were used and divided into six treatment groups: control with normal salt diet (0.5% NaCl, CON+NS), control with high-salt diet (4% NaCl, CON+HS), capsaicin pretreatment with high-salt diet (CAP+HS), capsaicin pretreatment plus high-salt diet and telmisartan (CAP+HS+T, 10 mg/kg/day), capsaicin pretreatment plus high-salt diet and perindopril (CAP+HS+P, 2 mg/kg/day), and capsaicin pretreatment plus high-salt diet and spironolactone (CAP+HS+S, 80 mg/kg/day). Treatment with different salt diets and drugs was started at 3 weeks of age and lasted 18 weeks. The specially made diet was obtained from Guangdong Medical Research Animal Center. These experiments were approved by the Animal Care Committee of Guangzhou Medical College.

2.2. Measurement of blood pressure

Tail-cuff systolic blood pressure and body weight were examined beginning at 3 weeks of age, biweekly for the first month, and then every 4 weeks until 19 weeks of age. At the end of the 18-week treatment period (21 weeks of age), the rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal). The right carotid artery was cannulated for direct blood pressure measurement. Mean blood pressure was calculated using the systolic and diastolic blood pressure values.

2.3. Morphometric measurements

After measuring the blood pressure through the carotid artery, the vessels were cleared of blood by perfusion as follows (Dickhout and Lee, 1997). An infusion cannula was placed in the abdominal aorta, distal to the origin of the renal artery and the superior mesenteric artery. An exit for the perfusate was cut into the portal vein. This allowed the perfusate, an oxygenated standard phosphate-buffered salt solution containing 1 μmol/L sodium nitroprusside, to clear the vasculature in the abdominal viscera. The arteries were perfused at a pressure of 100 cm H_2O and a flow rate of 1 ml/min per 100 g body weight for 5 min, resulting in the maximal relaxation of the arteries, and then perfused with the fixative composed of 10% formaldehyde-phosphate buffer solution. The thoracic aorta, main renal arteries and small mesenteric arteries were sampled and embedded in wax. Three-micron-thick cross-sections of these vessels were stained with hematoxylin and eosin. Morphometric measurement of the vessel-wall dimensions was carried out using a light microscope, a camera lucida and a digitizing pad, as described before (Lee and Triggle, 1986; Lee et al., 1989). The lumen diameter, media thickness, media-thickness-to-lumen-diameter ratios and cross-sectional area of these vessels were measured in these maximally relaxed vessels (Bund and Lee, 2003). Three sections from each rat were measured, and the average was calculated, that, in the final analyses, only one number from each rat was used.

2.4. Statistical analysis

All values were expressed as means±S.E.M. Statistical analyses were performed using SigmaStat 3.0 software. A repeated-measure analysis of variance (ANOVA) was used to test the difference in blood pressure among the groups at different ages. One-way ANOVA and Holm–Sidak test of subsequent pair-wise multiple comparison procedures were used to compare the physical characteristics of the rats and the structural differences of the blood vessels among different groups at 21 weeks of age. The differences were considered significant at $P<0.05$.

3. Results

Blood pressure was similar among all the groups before the dietary treatment. Two weeks after the initiation of the diet treatment (5 weeks of age), blood pressure became higher in the CAP+HS than in the other groups, and this

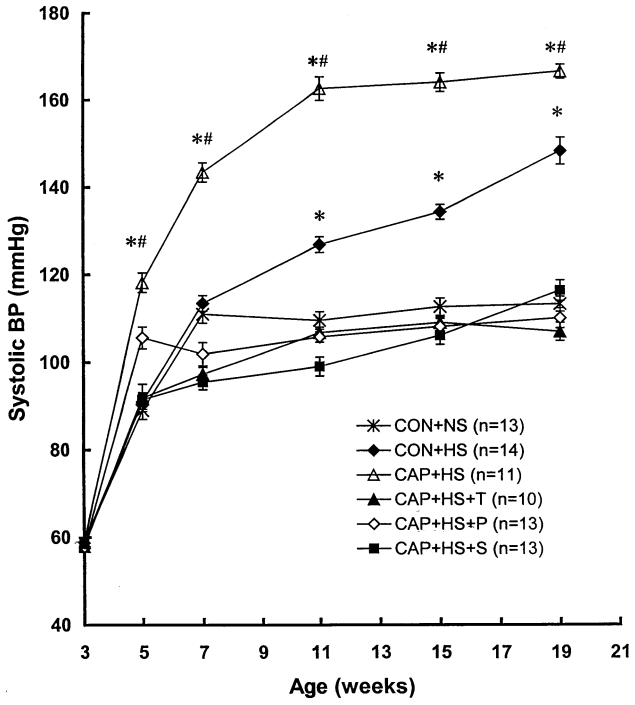

Fig. 1. Blood pressure profile of Wistar rats treated with capsaicin (CAP) given a high-salt (HS) diet and of control (CON) given a high-salt (HS) or normal salt diet (NS). Some of these rats were also treated with perindopril (+P), telmisartan (+T) or spironolactone (+S). Values are the mean±S.E.M. *P<0.01 compared with CON+NS and all the antihypertensive treatment groups; $^{\#}P$<0.01 compared with CON+HS.

difference was maintained until the end of the treatment period (Fig. 1). Beginning at 8 weeks after the initiation of the salt diet treatment (11 weeks of age), blood pressure also became higher in the CON+HS than in the CON+NS. Treatment with any of the three drugs was effective in preventing the development of hypertension in the CAP+HS group (Fig. 1). Direct measurement of mean blood pressure at the end of the study period confirmed the blood pressure results obtained with the tail-cuff method (Table 1).

Body weight was similar among all the groups before the initiation of the diets and drug treatment and at the end of the treatment period (Table 1). Left ventricular hypertrophy was present in the CAP+HS and CON+HS as compared with the CON+NS.

Values for the media thickness, media-thickness-to-lumen-diameter ratios and cross-sectional area in the thoracic aorta, renal artery and mesenteric artery were significantly higher in the CAP+HS groups than in the CON+HS and CON+NS groups (Table 2). Similarly, these values were higher in the CON+HS than in the CON+NS. There was a positive correlation between blood pressure and the cross-sectional area of the aorta, renal artery and mesenteric arteries (r≥0.92, P<0.01) among the CON+NS, CON+HS and CAP+HS groups.

The effect of the three different drugs on cardiovascular structural changes in CAP+HS was, different depending on the drugs. Perindopril was the only drug that prevented the development of left ventricular hypertrophy (Table 1). Treatment with perindopril or spironolactone prevented the development of medial hypertrophy in the aorta, renal artery and mesenteric artery, as indicated by the decrease in medial thickness, media-to-lumen ratio and cross-sectional area in these vessels as compared with CAP+HS or CON+HS (Table 2). Telmisartan was effective in preventing the development of medial hypertrophy only in the aorta and not in the renal and mesenteric arteries. Lumen diameter was not different among all the groups in any of the arteries.

4. Discussion

Our study showed that the prevention of hypertension development in a sensory deprivation model with antihypertensive agents targeted at the renin–angiotensin–aldosterone system was associated with the prevention of cardiovascular remodeling in these rats. However, the specific types of structural changes associated with the drug treatment were drug specific, with perindopril showing the most beneficial overall effects.

The mechanisms that may contribute to the increase in blood pressure in this hypertensive model include an impaired natriuretic response to a high salt intake (Wang

Table 1
Physical characteristics of the rats

	CON+NS (n=13)	CON+HS (n=14)	CAP+HS (n=11)	CAP+HS+T (n=10)	CAP+HS+P (n=13)	CAP+HS+S (n=13)
Body weight (g)						
3 weeks	49±1	50±1	52±1	51±1	49±1	49±1
21 weeks	387±5	366±7	360±8	365±6	361±5	363±5
LVW/BW ratio (mg/g)						
21 weeks	2.12±0.04	2.36±0.06*	2.47±0.06*	2.47±0.10	2.12±0.11	2.41±0.09
MAP (mm Hg)						
21 weeks	129±1	164±2†	181±2†,‡	122±2	125±2	132±3

Values are the mean±S.E.M. LVW/BW ratio=left ventricular weight to body weight ratio; MAP=mean arterial pressure.
* P<0.05 compared with CON+NS, and CAP+HS+P.
† P<0.05 compared with CON+NS and all the treatment groups.
‡ P<0.01 compared with CON+HS.

Table 2
Structural changes of the blood vessels

Vessel type	CON+NS (n=13)	CON+HS (n=14)	CAP+HS (n=11)	CAP+HS+T (n=10)	CAP+HS+P (n=13)	CAP+HS+S (n=13)
Aorta						
Lumen diameter (μm)	1709±30	1691±62	1816±55	1773±86	1674±41	1821±93
Medial thickness (μm)	134±4	161±10*	187±7*,†	140±8	127±6	121±6
M/L ratio	0.078±0.002	0.095±0.004*	0.103±0.004*	0.079±0.009	0.076±0.011	0.074±0.009
CSA (mm^2)	0.778±0.031	0.957±0.087*	1.179±0.073*,†	0.860±0.097	0.720±0.041	0.825±0.091
Renal artery						
Lumen diameter (μm)	474±20	495±30	490±56	477±39	472±33	462±54
Medial thickness (μm)	37±1	46±3*	52±2*,†	40±3	37±2	38±2
M/L ratio	0.078±0.004	0.092±0.006‡	0.106±0.015†,‡	0.084±0.01	0.079±0.007	0.082±0.020
CSA (mm^2)	0.059±0.004	0.078±0.005‡	0.089±0.011†,‡	0.07±0.01#	0.036±0.004	0.059±0.008
Mesenteric artery						
Lumen diameter (μm)	257±15	225±19	257±9	227±11	204±6	200±22
Medial thickness (μm)	22±2	31±2‡	36±4†,‡	29±3#	18±2	23±2
M/L ratio	0.094±0.01	0.156±0.02§	0.165±0.01§	0.13±0.01#	0.09±0.01	0.128±0.02
CSA (mm^2)	0.019±0.001	0.025±0.002‡	0.033±0.004†,‡	0.02±0.003	0.013±0.001	0.017±0.003

Values are the mean±S.E.M. M/L=media to lumen ratio, CSA=cross-sectional area.
* P<0.05 compared with CON+NS and all the treatment groups.
† P<0.01 compared with CON+HS.
‡ P<0.05 compared with CON+NS, CAP+HS+P, CAP+HS+S.
# P<0.05 compared with CON+HS+P.
§ P<0.05 compared with CON+NS, CAP+HS+P.

et al., 1998) and renin–angiotensin–aldosterone system (Huang and Wang, 2001a,b). In the studies by Wang et al., rats were given a high-salt diet concomitant antihypertensive treatment for only 2–3 weeks. It is not known if structural changes of the heart and blood vessels were involved in this model. Previous studies have shown that, with a few exceptions (e.g., vasodilators), antihypertensive agents that are effective in lowering blood pressure also affect the structure of the blood vessels (Lee, 1989). This is because, in humans and in various animal models, hypertension is associated with hypertrophy of the heart and blood vessels, thus, any blood pressure effect related to treatments will also affect cardiovascular changes. However, in this study, we found that, even with similar degree of blood pressure lowering by the three different drugs with different mode of actions, their effect on cardiovascular changes was different, depending on the drug.

Cardiac hypertrophy is a major risk factor for heart failure in hypertension. In this study, only perindopril was effective in preventing the development of cardiac hypertrophy in the CAP+HS rats. This is similar to the results obtained in the spontaneously hypertensive rats, where treatment of adult spontaneously hypertensive rats with perindopril was more effective in causing regression of cardiac hypertrophy than did treatment with an angiotensin AT_1 receptor antagonist (Gillies et al., 1998). In Dahl salt-sensitive rats, treatment with angiotensin converting enzyme inhibitors or angiotensin AT_1 receptor antagonist failed to prevent the development of hypertension, but these treatments significantly reduced cardiac hypertrophy in Dahl salt-sensitive rats (Hirawa et al., 1994; Sakata et al., 2004; Satoh et al., 2003). These results indicate that blood pressure lowering is not the only factor in either the prevention or reversal of cardiac hypertrophy in hypertension. Other mechanisms, such as the effects of the treatment on matrix metalloproteinases (Sakata et al., 2004), may be involved in this model. The blockade of angiotensin II production by an angiotensin converting enzyme inhibitor, or the inhibition of angiotensin AT_1 receptor antagonist, may lead to the production of a vasodilator, such as angiotensin (1–7), from angiotensin I through the action of angiotensin converting enzyme 2 (Carey, 2004; Yagil and Yagil, 2003), so that mechanisms other than the interference with actions of angiotensin II can be involved in producing the blood pressure lowering effects of perindopril in our model. In this study, we found that treatment with spironolactone prevented hypertension development but had an effect on preventing left ventricular hypertrophy, similar with the results obtained in deoxycorticosterone acetate/salt-induced hypertensive rats (Fujisawa et al., 2003). Taken together, these results, including ours, indicate that, in experimental hypertension models where a high-salt diet is involved, the development of cardiac hypertrophy is independent of blood pressure change.

The development of medial hypertrophy in this model is closely related to the blood pressure. This is because of the positive correlation found between blood pressure and the cross-sectional area of the aorta, renal artery and mesenteric arteries among the CON+NS, CON+HS and CAP+HS groups, and a higher value of medial thickness, media-to-lumen ratio and cross-sectional area in the three vessels from the CAP+HS than from the CON+HS. The effect of perindopril on vascular structural changes is also unique in that it is the only drug among the three drugs studied that prevented the development of medial hypertrophy in the aorta, renal and mesenteric arteries. This result is again

similar to those found in adult spontaneously hypertensive rats, where perindopril was more effective than an angiotensin AT_1 receptor antagonist was in altering the structural and functional changes of the mesenteric arteries (Gillies et al., 1998). Spironolactone was as effective as perindopril was in preventing vascular changes in the three vessels studied. This is probably because, in this model, sensory denervation is associated with an impairment of renal function, and treatment, with spironolactone improved renal functions (Huang and Wang, 2001b). In contrast, angiotensin AT_1 receptor antagonist telmisartan was the least effective because it prevented only the structural change in the aorta and not in the renal or mesenteric arteries. It is evident that vascular changes in this model are associated with other mechanisms besides AT_1-mediated responses.

In conclusion, we have shown that the prevention of hypertension in this model with drug treatment, which affected the renin–angiotensin–aldosterone system, is associated with the prevention of cardiovascular remodeling. However, the order of effectiveness in preventing structural changes of the heart and blood vessels was perindopril>spironolactone>telmisartan, suggesting that AT_1-mediated responses are probably not as important as other factors are in causing cardiovascular structural changes in this model.

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References

Bund, S.J., Lee, R.M., 2003. Arterial structural changes in hypertension: a consideration of methodology, terminology and functional consequence. J. Vasc. Res. 40, 547–557.

Carey, R.M., 2004. Angiotensin type-1 receptor blockade increases ACE 2 expression in the heart. Hypertension 43, 943–944.

Dickhout, J.G., Lee, R.M.K.W., 1997. Structural and functional analysis of small arteries from young spontaneously hypertensive rats. Hypertension 29, 781–789.

Fujisawa, G., Okada, K., Muto, S., Fujita, N., Itabashi, N., Kusano, E., Ishibashi, S., 2003. Na/H exchange isoform 1 is involved in mineralocorticoid/salt-induced cardiac injury. Hypertension 41, 493–498.

Gillies, L.K., Werstiuk, E.S., Lee, R.M., 1998. Cross-over study comparing effects of treatment with an angiotensin converting enzyme inhibitor and an angiotensin II type 1 receptor antagonist on cardiovascular changes in hypertension. J. Hypertens. 16, 477–486.

Hirawa, N., Uehara, Y., Kawabata, Y., Ohshima, N., Ono, H., Nagata, T., Gomi, T., Ikeda, T., Goto, A., Yagi, S., Omata, M., 1994. Mechanistic analysis of renal protection by angiotensin converting enzyme inhibitor in Dahl salt-sensitive rats. J. Hypertens. 12, 909–918.

Huang, Y., Wang, D.H., 2001a. Role of AT1 and AT2 receptor subtypes in salt-sensitive hypertension induced by sensory nerve degeneration. J. Hypertens. 19, 1841–1846.

Huang, Y., Wang, D.H., 2001b. Role of renin–angiotensin–aldosterone system in salt-sensitive hypertension induced by sensory denervation. Am. J. Physiol.: Heart Circ. Physiol. 281, H2143–H2149.

Jones, D.W., 2004. Dietary sodium and blood pressure. Hypertension 43, 932–935.

Lee, R.M.K.W., 1989. Structural and functional consequence of antihypertensive treatments on Blood Vessels. In: Lee, R.M.K.W. (Ed.), Blood Vessels Changes in Hypertension: Structure and Function, vol. I. CRC Press, Boca Raton, FL, pp. 163–190.

Lee, R.M.K.W., Triggle, C.R., 1986. Morphometric study of mesenteric arteries from genetically hypertensive Dahl strain rats. Blood Vessels 23, 199–224.

Lee, R.M.K.W., Richardson, M., McKenzie, R., 1989. Vascular changes associated with deoxycorticosterone-NaCl-induced hypertension. Blood Vessels 26, 137–156.

Sakata, Y., Yamamoto, K., Mano, T., Nishikawa, N., Yoshida, J., Hori, M., Miwa, T., Masuyama, T., 2004. Activation of matrix metalloproteinases precedes left ventricular remodeling in hypertensive heart failure rats: its inhibition as a primary effect of angiotensin-converting enzyme inhibitor. Circulation 109, 2143–2149.

Satoh, S., Ueda, Y., Suematsu, N., Oyama, J., Kadokami, T., Sugano, M., Yoshikawa, Y., Makino, N., 2003. Beneficial effects of angiotensin-converting enzyme inhibition on sarcoplasmic reticulum function in the failing heart of the Dahl rat. Circ. J. 67, 705–711.

Wang, D.H., Li, J., 1999. Antihypertensive mechanisms underlying a novel salt-sensitive hypertensive model induced by sensory denervation. Hypertension 33, 499–503.

Wang, D.H., Li, J., Qiu, J., 1998. Salt-sensitive hypertension induced by sensory denervation: introduction of a new model. Hypertension 32, 649–653.

Yagil, Y., Yagil, C., 2003. Hypothesis: ACE2 modulates blood pressure in the mammalian organism. Hypertension 41, 871–873.

Zeng, Z.H., Luo, B.H., Gao, Y.J., Su, C.J., He, C.C., Li, N., Lee, R.M., 2004. Arterial structural changes in hypertensive rats induced by sensory denervation with capsaicin. Clin. Exp. Pharmacol. Physiol. 31, 502–505.