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Proliferation of aortic smooth muscle cells and renin-angiotensin system in SHR rats[1]

WANG Xiang-Yu[2], WU Ke-Gui, JIN Xue-Qing, WANG Hua-Jun
(*Hypertension Division, the First Affiliated Hospital, Fujian Medical University, Fuzhou 350005, China*)

KEY WORDS inbred SHR rats; inbred WKY rats; vascular smooth muscle; cultured cells; renin-angiotensin system; captopril; saralasin

AIM: To study the relationship between the enhanced proliferation and renin-angiotensin system (RAS) of aortic smooth muscle cells (ASMC) from SHR rats. **METHODS**: To measure the effects of angiotensin II (Ang), captopril (Cap), saralasin (Sar) on proliferation, Ang and angiotensin converting enzyme (ACE) levels in cultured ASMC from WKY and SHR rats. **RESULTS**: Ang was a bifunctional growth factor, which induced SHR ASMC hyperplasia in 2 % FCS-RPMI 1640 medium, but not in serum free (SF)-medium. SHR ASMC had stronger proliferative ability compared with WKY while SHR ASMC RAS was activated. Enhanced proliferation of SHR ASMC and ACE activity were obviously inhibited by long-term treatment (4-wk) of both Cap and Sar, while Ang content decreased in Cap treatment group and increased in Sar treatment group. The antiproliferative effect of Cap and Sar on SHR ASMC was stronger than that on WKY. SHR, WKY ASMC RAS were not influenced by short-term (24 h) treatment of Cap. **CONCLUSION**: Long-term treatment of Cap and Sar suppressed SHR ASMC growth through inhibition of Ang generation or blockade of Ang binding to its receptor.

The endocrine renin-angiotensin system (RAS) plays a short-term role in the regulation of cardiovascular system. But the local RAS plays an important role in the long-term regulation of cardiovascular homeostasis[1]. This is achieved by the sustained activation of local angiotensin and the secondary structural changes of the cardiovascular system[2]. The presence of a local tissue RAS is independent of the circulating RAS. Angiotensin II (Ang) is a bifunctional growth factor that activated both proliferative and antiproliferative pathways to induce aortic smooth muscle cells (ASMC) hypertrophy or hyperplasia[3,4], depending on the cellular melieu and intrinsic genetic characters. Angiotensin converting enzyme inhibitors (ACEI) captopril (Cap) inhibits the conversion of angiotensin I to Ang and decreases the tissue Ang concentration while Ang-receptor antagonist saralasin (Sar) blocks Ang binding to its receptor, both of which are associated with a regression of cardiovascular hypertrophy[5,6]. Cardiovascular hypertrophy has been attributed to the effects of multifactor, such as blood pressure, sympathetic nervous activity, endogenous growth factors and so on. To eliminate the interaction of the multifactor *in vivo* and study the paracrine or autocrine mechanism of Ang-mediated remodelling of the cardiovascular system, it would be helpful to compare the different effects of Ang on ASMC from SHR and WKY in different cultured conditions and analyze the direct effects of short- and long-term treatment of Cap and Sar on proliferation and RAS of cultured ASMC.

MATERIALS AND METHODS

Cell culture ASMC were obtained by an explant method[7] from aortae of 20-wk-old ♂ SHR and WKY rats (General grade, № 23-021, Shanghai Institute of Hypertension). They were cultured at 37 ℃ in 5 % CO_2 atmosphere in RPMI-1640 medium with 15 % fetal calf serum (FCS, Hangzhou Si Ji Qing Corp). Cells were routinely used from the 3rd - 5th passage. Trypsinized cells were plated in 24-well plates to grow in RPMI-1640 containing 15 % FCS for 24 h, the culture medium was changed to RPMI-1640 without FCS for 48 h to establish quiescence.

Experiment groups Ang groups: Quiescent cells were divided into a) control group: 2 % FCS; b) 2 % FCS + Ang (0.001 - 1 $\mu mol \cdot L^{-1}$, Sigma); c) 2 % FCS + Ang (0.1 $\mu mol \cdot L^{-1}$) + Sar (0.01 - 1 $\mu mol \cdot L^{-1}$, Sigma). Cells were

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[2] Corresponding author. Phn 86-591-335-7199, ext 756. Fax 86-591-3318-716.
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labeled with [^{3}H] thymidine ([^{3}H] TdR) for 30 h. Sar were added 30 min before the addition of Ang, and coincubated throughout the experiment.

Cells were divided into a) 2 % FCS; b) 2 % FCS + Ang (1 μmol·L^{-1}); c) 15 % FCS. Cells were incubated for 4 d without or with Sar 10 μmol·L^{-1}. Ang and Sar were added every 24 h. After 4 d, cells was recovered using trypsin/egtazic acid and counted by hemocytometer.

2 % FCS control group was replaced by SF to repeat previous procedure.

Cap and Sar groups: Randomly cycling ASMC were divided into: a) control group: 15 % FCS; b) 15 % FCS + Cap (0.01, 1, 100 μmol·L^{-1}); c) 15 % FCS + Sar (0.1, 1, 10 μmol·L^{-1}). Cap or Sar was added every 24 h for 4-wk to ASMC when ASMC grew from the explant for long-term treatment. Cap was only added for 24 h for short-term treatment. The ASMC were labeled with [^{3}H]TdR for 24 h.

Measurement methods [^{3}H] TdR incorporation was determined by liquid scintillation spectrometry (FJ2115) after precipitation with ice-cold trichloroacetic acid (TCA, 5 % wt/vol): $DT = (t_1 - t_2) \times \lg 2/(\lg N_1 - \lg N_2)$, where N_1 and N_2 were the cell numbers at time t_1 and t_2, t_1, and t_2 were d 6 and d 4 respectively.

Ang and ACE determinations: ASMC were plated $3 \times 10^7 - 5 \times 10^7$ cells·L^{-1} to grow for 3 – 5 d. Culture medium was also plated as control. The cell number was counted before determination of Ang and ACE levels. Ang and ACE activity were quantitated in cultured ASMC and media by radioimmunoassay and spectrophotometry respectively[8-10].

Cultured cells were scraped from the flask. The cell suspension was spun at 500 × g at 4 ℃ for 5 min. Pellets were resuspended in 1 mL PBS, and stored at −80 ℃ for at least 1 month to allow for inactivation of Cap before assay for ACE activity[10]. After the −80 ℃ inactivation period, samples were sonicated and spun at 14 000 × g at 4 ℃ for 20 min. The pH of PBS used for determinations of Ang and ACE were 7.4 and 8.3 respectively.

Statistical analysis Data were expressed as $\bar{x} \pm s$ and compared with t test and Hotelling T^2 test by SPSS/PC. Each group at time point was performed in triplicate.

RESULTS

Ang-induced hyperplasia of SHR ASMC with 2 % FCS Ang-induced [^{3}H]TdR incorporation was much higher in SHR ASMC than those in WKY ($P < 0.01$). [^{3}H] TdR incorporation of SHR and WKY ASMC were increased 3.3 and 2.2 times by Ang 1 μmol·L^{-1}, respectively ($P < 0.01$) (Fig 1). Ang 1 μmol·L^{-1} yielded a 73 % ± 23 % increase in SHR cell number, but had no effect in WKY (Tab 1).

Ang-induced proliferation of these cells was

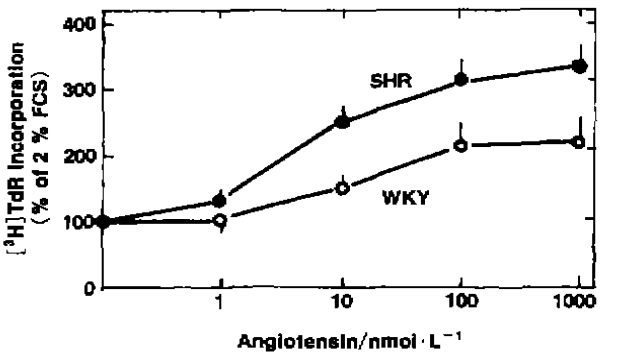

Fig 1. Ang stimulated [^{3}H] TdR incorporation in ASMC from SHR and WKY in 2 % FCS. Basal level of [^{3}H] TdR incorporation in ASMC from WKY and SHR without Ang was 55 ± 4, 69 ± 10 Bq/10^5 cells, respectively. $n = 8$ rats, $\bar{x} \pm s$. Hotelling T^2 test, $P < 0.01$, SHR *vs* WKY.

Tab 1. Effects of Sar 10 μmol·L^{-1} on proliferation of ASMC induced by Ang 1 μmol·L^{-1}. Cells were seeded at the same initial density and incubated for 4 d. $n = 8$ rats, $\bar{x} \pm s$. [a]$P > 0.05$ *vs* WKY SF; [d]$P > 0.05$ *vs* WKY 2 % FCS; [g]$P > 0.05$ *vs* WKY Ang + 2 % FCS; [j]$P > 0.05$ *vs* SHR SF; [e]$P < 0.01$ *vs* SHR 2 % FCS; [r]$P < 0.01$ *vs* SHR Ang + 2 % FCS.

	10^{-3} × Cells/well	
	WKY	SHR
2 % FCS	52 ± 6	60 ± 6
Ang + 2 % FCS	54 ± 9[d]	103 ± 12[e]
15 % FCS	118 ± 13	243 ± 14
2 % FCS + Sar	51 ± 6[d]	42 ± 8[e]
Ang + 2 % FCS + Sar	52 ± 8[g]	43 ± 5[r]
15 % FCS + Sar	118 ± 13	231 ± 19
SF	29 ± 2	31 ± 2
Ang + SF	30 ± 2[a]	32 ± 2[j]
Ang + SF + Sar	30 ± 2[a]	31 ± 2[j]

inhibited by Sar with concentration 10 times that of Ang. [^{3}H]TdR incorporations of SHR and WKY ASMC induced by Ang 0.1 μmol·L^{-1} were inhibited by Sar 1 μmol·L^{-1} and the inhibition rates were 67 % ± 3 %, 45 % ± 10 %, respectively (Fig 2). The cell number of SHR ASMC induced by Ang 0.1 μmol·L^{-1} was also inhibited by 58 % ± 2 % by Sar 1 μmol·L^{-1} ($P < 0.01$) (Tab 1).

Ang-induced hypertrophy of SHR ASMC in SF-medium [^{3}H] TdR incorporations of SHR and WKY ASMC were increased 1.9 and 1.3 times by Ang 0.1 μmol·L^{-1}, respectively ($P < 0.01$). [^{3}H]TdR incorporations of SHR and WKY ASMC induced by

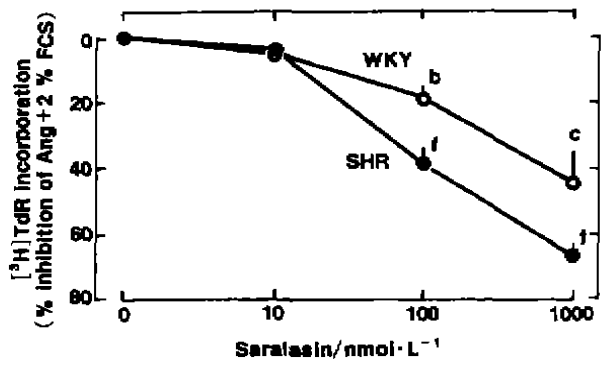

Fig 2. Effects of Sar on [³H]TdR incorporation in ASMC with Ang 0.1 μmol·L⁻¹ in 2 % FCS medium. Basal level of [³H]TdR incorporation in ASMC from WKY and SHR without Sar was 118 ± 22, 214 ± 20 Bq/10⁵ cells respectively. n = 8 rats, $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* WKY control; $^fP < 0.01$ *vs* SHR control.

Ang 0.1 μmol·L⁻¹ were also inhibited 34 % ± 6 % and 16 % ± 9 % by Sar 1 μmol·L⁻¹, respectively ($P < 0.01$) (Tab 2). The cell number of both SHR and WKY cells remained unchanged (Tab 1).

Tab 2. Effects of Sar 1 μmol·L⁻¹ on [³H] TdR incorporation of ASMC induced by Ang 0.1 μmol·L⁻¹. n = 8 rats, $\bar{x} \pm s$. $^cP < 0.01$ *vs* WKY SF; $^fP < 0.01$ *vs* WKY Ang + SF; $^iP < 0.01$ *vs* SHR Ang + SF.

	[³H]TdR incorporation, Bq/10⁵ cells	
	WKY	SHR
SF	21 ± 2	21 ± 2
Ang + SF	27 ± 3^c	40 ± 5^f
Ang + SF + Sar	22 ± 3^f	24 ± 2^i

Long-term treatment of Cap and Sar on proliferation, Ang and ACE levels [³H] TdR incorporation was higher in SHR ASMC than in WKY ($P < 0.01$). DT for SHR ASMC was 30.0 ± 1.4 h and for WKY 47.5 ± 2.5 h ($P < 0.01$), showing accelerated cell division by SHR ASMC. The antiproliferative effects of Cap and Sar on SHR ASMC were stronger than that on WKY. Cap 0.1 mmol·L⁻¹ caused 31 % ± 4 % and 17 % ± 9 % decreases in SHR and WKY ASMC [³H]TdR incorporation and prolonged DT for 13 and 6 h ($P < 0.01$), respectively. The levels of Ang and ACE in SHR ASMC and medium were much higher than those in WKY ($P < 0.01$), showing that SHR ASMC RAS was activated. Ang content and ACE activity in ASMC were decreased for 25 % ± 9 %, 27 % ± 13 % by Cap 0.1 mmol·L⁻¹, respectively, while Ang in culture was also decreased for 34 % ± 12 %. Cap 0.1 mmol·L⁻¹ only decreased Ang in WKY ASMC, Ang in medium and ACE in ASMC were unchanged.

[³H]TdR incorporation of SHR ASMC was inhibited 20 % ± 3 % by Sar 10 μmol·L⁻¹ ($P < 0.05$) while the DT prolonged for 7 h. Only Sar 10 μmol·L⁻¹ inhibited [³H]TdR incorporation of WKY ASMC 14 % ± 3 % while DT prolonged for 3 h ($P < 0.05$). Sar 10 μmol·L⁻¹ stimulated the synthesis and secretion of Ang and decreased ACE of SHR ASMC ($P < 0.05$). Sar 10 μmol·L⁻¹ also stimulated the synthesis and secretion of Ang of WKY ASMC ($P < 0.05$), but had no effects on WKY ASMC ACE activity (Tab 3).

Short-term treatment of Cap on the proliferation and Ang and ACE levels Only Cap 0.1 mmol·L⁻¹ inhibited [³H] TdR incorporation of SHR ASMC by 10 % ± 2 % ($P < 0.05$). Cap had no effects on [³H] TdR incorporation of WKY ASMC. Cap also had no effects on Ang and ACE levels of ASMC from SHR and WKY rats (Tab 4).

DISCUSSION

Ang-induced hypertrophy or hyperplasia of SHR ASMC in SF- or 2 % FCS-medium In this study we explored the possibility that Ang might exert a mitogenic action on ASMC and therefore contribute to the development of hypertensive arteriopathy. Such a contribution would be more important in animal with hyperresponsive cells. Several cells from SHR have been shown to present such a hyperresponsiveness to various agonist[11]. We therefore compared the action of Ang on ASMC from WKY and SHR. The results demonstrated that [³H] TdR incorporation of SHR, WKY ASMC could always be promoted by Ang under the same cultured conditions. However, the cell number of both strains of cells remained unchanged in SF-medium. But in 2 % FCS-medium, Ang induced SHR ASMC hyperplasia. Therefore, it was reasonable to hypothesize that Ang served as partial growth factor for SMC, initiating the increased cell mass associated with cell cycle progression and in some

Tab 3. Effects of long-term treatment of Cap and Sar on [^{3}H]TdR incorporation, doubling time, Ang content, and ACE activity of random cycling ASMC from WKY and SHR. $n = 8$ rats, $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* WKY control; $^eP < 0.05$, $^fP < 0.01$ *vs* SHR control.

		[^{3}H]TdR incorporation (Bq/10^5 cells)		Doubling time (h)		Ang in ASMC (pg/10^6 cells)		Ang in medium (pg/10^6 cells)		ACE activity (U/10^6 cells)	
		WKY	SHR	WKY	SHR	WKY	SHR	WKY	SHR	WKY	SHR
Control		393 ± 49	703 ± 63^c	47.5 ± 2.5	30.0 ± 1.4^c	102 ± 17	208 ± 36^c	44 ± 16	102 ± 18^c	46 ± 14	66 ± 14^c
Cap, μmol·L^{-1}	0.01	369 ± 53	618 ± 58^f	47.8 ± 1.9	34.8 ± 2.5^f	99 ± 14	203 ± 36	41 ± 12	99 ± 14	48 ± 15	63 ± 19
	1	365 ± 34	584 ± 75^f	49.2 ± 2.2	40.8 ± 2.2^f	97 ± 20	179 ± 31^e	42 ± 18	81 ± 20^c	46 ± 15	52 ± 14^e
	100	325 ± 41^b	484 ± 43^f	53.1 ± 2.3^c	43.6 ± 2.4^f	84 ± 13^b	144 ± 32^e	37 ± 11	56 ± 14^f	43 ± 17	48 ± 13^e
Sar, μmol·L^{-1}	0.1	393 ± 47	697 ± 73	48.7 ± 1.9	29.9 ± 1.4	110 ± 14	210 ± 39	40 ± 12	107 ± 19	47 ± 18	65 ± 17
	1	370 ± 33	620 ± 69^e	47.8 ± 2.7	32.0 ± 1.8^e	105 ± 14	243 ± 33^e	46 ± 11	128 ± 19^e	43 ± 11	58 ± 10
	10	339 ± 44^b	542 ± 73^f	50.6 ± 3.0^b	37.8 ± 1.8^f	121 ± 19^b	251 ± 33^e	60 ± 14^b	130 ± 19^e	40 ± 16	53 ± 11^e

Tab 4. Effects of short-term treatment of Cap on [^{3}H]TdR incorporation, Ang content, and ACE activity of random cycling ASMC from WKY and SHR. $n = 8$ rats, $\bar{x} \pm s$. $^aP > 0.05$ *vs* WKY control; $^dP > 0.05$, $^eP < 0.05$ *vs* SHR control.

Captopril (μmol·L^{-1})	[^{3}H]TdR incorporation (Bq/10^5 cells)		Ang in ASMC (pg/10^6 cells)		Ang in medium (pg/10^6 cells)		ACE in SMC (U/10^6 cells)	
	WKY	SHR	WKY	SHR	WKY	SHR	WKY	SHR
Control	393 ± 49	703 ± 63	102 ± 17	210 ± 36	44 ± 16	102 ± 18	46 ± 14	66 ± 14
0.01	400 ± 63	697 ± 69	–	–	–	–	–	–
1	377 ± 67	677 ± 73	–	–	–	–	–	–
100	347 ± 57^a	631 ± 57^e	120 ± 29^a	190 ± 38^d	40 ± 14^a	105 ± 21^d	44 ± 14^a	70 ± 17^d

cases DNA replication and cell division. In light of the preceding, it might be concluded that the 2 % FCS-contained micro-growth factor such as PDGF, EGF, FGF might be sufficient to suppress Ang-induced antiproliferative pathway in SHR ASMC, but not in WKY; Minimal amount of second messengers contained in 2 % FCS-medium were enough to trigger SHR ASMC proliferation, but not to WKY.

The effects of Cap and Sar on abnormal proliferation and Ang, ACE levels of SHR ASMC The cultured ASMC from SHR stimulated by growth factors of FCS proliferatived more rapidly than cells from WKY. This result suggested that ASMC from SHR might have an intrinsic genetic abnormality in the control of ASMC proliferation. ASMC was capable of synthesizing and secreting Ang and ACE while SHR ASMC RAS was activated. The existence of mRNA for angiotensinogen and renin in aorta had been reported by a number of laboratories[12,13]. All these suggested independent RAS existed in ASMC. Long-term treatment of Cap and Sar suppressed SHR ASMC growth through inhibition of Ang generation or blockade of Ang binding to its receptor. The antiproliferative action of Cap and Sar on SHR ASMC was stronger than on WKY ASMC. That may be related to the activated RAS of SHR ASMC. Suppression of SHR ASMC growth by Cap 0.01 μmol·L^{-1} was not mediated by inhibition of the RAS, activation of kinin-prostaglandin axis was probably responsible for this[14]. Both Cap and Sar could also suppress WKY ASMC growth, suggesting Ang may be an important nutritious factor of cardiovascular tissue and participate in its normal growth in normotensive rats as well. The results that Ang levels in ASMC were increased by Sar treatment, which was caused by feedback regulation and suppression of Ang catabolism via receptor sequestration, was similar to the report of Campbell[15]. There was no indication that the increased Ang levels were seriously affecting the ability of Ang-receptor antagonist to lower blood pressure and reverse cardiovascular hypertrophy.

Short-term treatment of Cap did not have the effects of long-term treatment of Cap. Short-term treatment of Cap did not inhibit Ang content and ACE

activity of ASMC from SHR and WKY, showing that the suppression of tissue RAS required long-term treatment with ACEI.

REFERENCES

1 Dzau VJ. Short- and long-term determinants of cardiovascular function and therapy: contributions of circulating and tissue renin-angiotensin systems.
J Cardiovasc Pharmacol 1989; 14 Suppl 4: S1 – 5.

2 Black MJ, Bertram JF, Campbell JH, Campbell GR. Angiotensin II induces cardiovascular hypertrophy in perindopril-treated rats.
J Hypertens 1995; 13: 683 – 92.

3 Naftilan AJ, Pratt RE, Dzau VJ. Induction of platelet-derived growth factor A-chain and c-*myc* gene expressions by angiotensin II in cultured rat vascular smooth muscle cells.
J Clin Invest 1989; 83: 1419 – 24.

4 Dzau VJ. Vascular renin-angiotensin system and vascular protection. J Cardiovasc Pharmacol 1993; 22 Suppl 5: S1 – 9.

5 Hu WY, Chen DG, Chen SC, Jin XQ, Wang HJ.
Effect of chronic captopril treatment on circulating and tissue renin-angiotensin system in SHR rats.
Acta Pharmacol Sin 1996; 17: 507 – 12.

6 Mizuno K, Tani M, Hashimoto S, Niimura S, Sanada H, Watanabe H, *et al*. Effects of losartan, a nonpeptide angiotensin II receptor antagonist, on cardiac hypertrophy and the tissue angiotensin II content in spontaneously hypertensive rats.
Life Sci 1992; 51: 367 – 74.

7 Chamley-Campbell J, Campbell GR, Ross R. The smooth muscle cell in culture. Physiol Rev 1979; 59: 1 – 61.

8 Shimamoto K, Ishida H, Nakahashi Y, Nishitani T, Hosoda S, Yokoyama T, *et al*. A very sensitive direct radioimmunoassay system for plasma angiotensin II and its clinical application in various hypertensive diseases.
Jpn Circ J 1984; 48: 1228 – 35.

9 Holmquist B, Bünning P, Riordan JF. A continuous spectrophotometric assay for angiotensin converting enzyme.
Anal Biochem 1979; 95: 540 – 8.

10 King SJ, Oparil S. Converting-enzyme inhibitors increase converting-enzyme mRNA and activity in endothelial cells.
Am J Physiol 1992; 263 (4 pt 1): C743 – 9.

11 Hamet P, Hadrava V, Kruppa U, Tremblay J. Vascular smooth muscle cell hyperresponsiveness to growth factors in hypertension.
J Hypertens 1988; 6 Suppl 4: S36 – 9.

12 Campbell DJ, Habener JF. Angiotensinogen gene is expressed and differentially regulated in multiple tissues of the rat.
J Clin Invest 1986; 78: 31 – 9.

13 Re R, Fallon JT, Dzau V, Ouay SC, Haber E.
Renin synthesis by canine aortic smooth muscle cells in culture.
Life Sci 1982; 30: 99 – 106.

14 Campbell DJ, Kladis A, Duncan A-M. Effects of converting enzyme inhibitors on angiotensin and bradykinin peptides.
Hypertension 1994; 23: 439 – 49.

15 Campbell DJ, Kladis A, Valentijn AJ. Effects of losartan on angiotensin and bradykinin peptides and angiotensin-converting enzyme. J Cardiovasc Pharmacol 1995; 26: 233 – 40.

自发性高血压大鼠主动脉平滑肌细胞异常增殖和局部肾素-血管紧张素系统

王向宇[1]，吴可贵，晋学庆，王华军 （福建医科大学附属第一医院高血压研究所，福州 350005，中国）

关键词 近交 SHR 大鼠；近交 WKY 大鼠；血管平滑肌；培养的细胞；肾素-血管紧张素系统；卡托普利；沙拉新

目的：探讨 SHR 大鼠主动脉平滑肌细胞（ASMC）异常增殖和肾素-血管紧张素系统（RAS）的关系. **方法**：测定血管紧张素 II（Ang）、卡托普利（Cap）、沙拉新（Sar）对培养的 SHR、WKY ASMC 增殖和 Ang、血管紧张素转化酶（ACE）的影响. **结果**：Ang 在 2 % 血清培养基中可刺激 SHR ASMC 增生. SHR ASMC 分裂增殖能力比 WKY 强，SHR ASMC RAS 处于高功能状态. Cap 长期（4 周）干预显著抑制 SHR ASMC 异常增殖和 Ang、ACE 活性，Sar 长期干预同样抑制 SHR ASMC 的增殖和 ACE 活性，但 Ang 水平反而升高. Cap 短期（24 小时）干预不影响两种大鼠 ASMC RAS. **结论**：Cap 和 Sar 长期干预通过减少 SHR ASMC Ang 生成或阻断 Ang 和特异受体结合，抑制其异常增殖.