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离子通道中记忆性的存在[1]

R966

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目的：探索血管平滑肌培养细胞 Ca^{2+} 依赖性 K^+ 通道和肾上腺髓质瘤无性繁殖细胞电压依赖性 K^+ 通道的斑片钳记录中记忆的存在性。**方法**：基于原数字化信号或对应通道开关的0－1序列而非基于持续时间的序列，计算样本自相关函数。**结果**：样本自相关函数具有随时间跨度下降的趋势，对重复观察有稳定性，对不同处理有敏感性。**结论**：某些单离子通道可能存在记忆性，作为信号的一种内在特性，独立于对有限时间分辨力引起的疏漏观察所作的任何外在假定。

关键词 离子通道；记忆；胸主动脉；血管平滑肌；PC12细胞；神经生长因子；钾

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Inhibition of left ventricular hypertrophy and expression of proto-oncogenes c-*myc* other than c-*fos* in myocardium by early captopril treatment in SHR rats[1]

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AIM: To explore the mechanisms by which angiotensin converting enzyme inhibitor (ACEI) prevents the development of left ventricular hypertrophy (LVH). **METHODS**: Captopril (Cap 100 $mg \cdot kg^{-1} \cdot d^{-1}$) was given orally to ♀ spontaneously hypertensive rats from intrauterine period to 16 wk of age. Experiments were performed at 40 wk of age. SBP, left ventricular weight to body weight ratio (LVW/BW) were assessed. The levels of c-*myc* and c-*fos* mRNA in the left ventricle were measured by Northern blot. **RESULTS**: Early-onset Cap therapy significantly decreased SBP. After discontinuance of treatment for 24 wk, SBP of SHR_{cap} was still maintained at a lower level. LVW/BW in SHR_{cap} was markedly reduced. The expression of myocardial c-*myc* mRNA was decreased by

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72 % in SHR_{cap} compared with that in the untreated SHR, but the expression of myocardial c-*fos* mRNA was not different between the untreated SHR, SHR_{cap}, and WKY rats. **CONCLUSION**: Early Cap treatment may permanently prevent the development of hypertension, inhibit LVH. Furthermore, the prevention of LVH is associated with a decrease in c-*myc* mRNA levels, and the development and regression of left ventricular hypertrophy may be irrelevant to c-*fos* expression.

KEY WORDS left ventricular hypertrophy; proto-oncogenes proteins c-*myc*; proto-oncogene protein c-*fos*; captopril; inbred SHR rats

Left ventricular hypertrophy (LVH) may respond to various stimuli such as mechanical load, α_1-adrenergic receptor agonists[1], myotrophin[2], and angiotensin II (AII)[3]. They elicited the "immediate early genes" eg, proto-oncogenes, c-*fos* and c-*myc* transcript, and "late responsive genes" eg, fetal contractile protein genes for β-myosin heavy chain and α-skeletal actin expression, subsequently leading to increase in fetal contractile protein synthesis and myocardial cell size, as well as LVH development. Some authors suggested that changes in expression of c-*myc* and c-*fos* might be a part of a sequence of events resulting in myocyte hypertrophy[4].

Angiotensin converting enzyme inhibitor (ACEI), Cap, lowered blood pressure and inhibited cardiac hypertrophy. So far, no study on the relation between the change in cardiac proto-oncogene expression and LVH inhibition by chronic administration of Cap has been reported. The purpose of this study is to investigate the changes in cardiac c-*fos* and c-*myc* mRNA levels during LVH inhibited by early Cap treatment, in the hope of obtaining an understanding of the mechanism by which Cap inhibits LVH.

MATERIALS AND METHODS

Chemicals and DNA probes Chemicals for RNA extraction and standard Chemicals used in the experiments were purchased from Sigma Co Agarose, Random primer Kit and T_4 polynucleotide kinase were purchased from Promega Co (Madison Wi, USA). Mops and nylon membranes were bought from Boehinger Co (Germany). Nuctrap™ Push Columns came from Stratagene Co (LaJolla CA), [α-^{32}P] dCTP and [γ-^{32}P]dATP were bought from Beijing Furei Co. Rat c-*fos* and c-*myc* cDNA probes were obtained from Institute of Cardiovascular Basic Research, Beijing Medical University. 18S Oligonucleotide probe was a kind gift from Dr Yiu-Fai CHEN (University of Alabama, Birmingham AL, USA). Other chemicals were either AR or molecular biology grade. Cap was bought from Sino-American Shanghai Squibb Pharmaceutical, Ltd.

Rats and treatment All rats were offsprings of breeders derived from Shanghai Institute of Hypertension. Once parental ♂ and ♀ SHR of 16 wk old were put together in cages, Cap (100 $mg \cdot kg^{-1} \cdot d^{-1}$,) was given once a day orally in mixture with small amount of milk powder. The ♀ SHR were maintained on this dosage throughout pregnancy and lactation. The ♂ weaned pups continued to be treated until 16 wk of age. ♂, age-matched untreated SHR and WKY controls were given only milk powder. The rats were housed in groups of 3—4 each cage at constant temperature 22±3 ℃, humidity 60±5 % and a 12 h light/dark cycle. Water and standard laboratory rat chow were consumed *ad lib*. Experiments were performed on 40-week-old ♂ rats.

Blood pressure measurement Systolic blood pressure (SBP) and heart rate (HR) were measured using tail-cuff technique (MRB-Ⅱ A computer control sphygmomanometer for rats, Shanghai Institute of Hypertension). After drug withdrawal, SBP in Cap-treated SHR (SHR_{cap}), untreated SHR, and WKY, were measured every 6 wk.

Measurement of left ventricle weight (LVW) to body weight (BW) ratio After weighed, the rats were decapitated. The hearts excised, great vessels, atria and right ventricular free walls were removed. The ventricles with the interventricular septum were

determined. LVH was assessed by LVW/BW.

RNA extraction and Northern blot A block of left ventricles was immediately homogenized by using a polytron homogenizer on ice. Subsequent extraction of total cellular RNA followed the acid guanidinium thiocyanate-phenol-chloroform procedure[5]. Total RNA was dissoloved in 0.1 % DEPC-treated water containing 1.5×10^6 U RNasin·L^{-1}. The quantity and purity of the RNA were determined by measuring the A_{260} and A_{280}. Total RNA was separated by elertrophoresis through agarose gel, and transferred to nylon membranes by capillary blotting. The quantity of RNA in each track was verified by ethidum bromide staining before and after the transfer. The RNA was fixed to the membrane by the exposure to uv rays. The membrane was prehybridized at 42 ℃ for at least 2 h. Hybridization was performed at 42 ℃ for 24 h using the same buffer containing the appropriate ^{32}P-labeled DNA probe prepared by random-priming precedure. [α-^{32}P]dCTP was separated from the ^{32}P-labeled probe by Nuctrap™ Push Column. Specific radioactivities were 1.9×10^{15} dpm/g DNA. Unhybridized probes were removed from the nylon membrane with SSC/SDS. The membranes were then exposed to Fuji X-ray film (Fuji-photo-film, Japan) for 2—3 d (up to 7 d for c-*myc*) with an intensifying screen at −70 ℃. After autoradiography, the membranes were washed in 0.1 % SSC/0.1 % SDS for at 95 ℃ 3—5 min. To control the possible sample variability, the membranes were rehybridized with a 18S oligonucleotide probe as an internal standardization. The probe was radiolabeled by using T_4 polynucleotide kinase and [γ-^{32}P] ATP. Relative amounts of RNA were determined by densitometric scanner (Beckman Appraise™ Densitometer, USA). The densitometric scores of specific mRNA were normalized by that of 18S rRNA.

Statistical analysis Data were expressed as $\bar{x}\pm s$. ANOVA with Newman Kuels procedure was used to evaluate the differences between the 3 groups.

RESULTS

1 Cap prevented the development of hypertension in SHR, even after removal of treatment.

2 Cap sustainedly inhibited LVH, even after withdrawal of treatment.

3 Cap reduced cardiac expression of c-*myc* proto-oncogene. Inhibition of LVH was associated with the attenuation of cardiac c-*myc* expression.

4 Cap did not change cardiac c-*fos* expression. The expression of cardiac c-*fos* was not essential for the development and inhibition of cardiac hypertrophy.

At 16 wk of age, when the medication was removed, SBP of SHR_{cap}, untreated SHR and WKY were 19.5±1.2, 27.2±1.3, 16.8 ± 0.8 kPa, respectively. SHR_{cap} had a significant decrease in SBP (7.7±1.2 kPa) compared with untreated SHR. After withdrawal of treatment, SBP in SHRcap slightly rose with age, but still maintained a relatively lower level than that of age-matched SHR (20.9 ±1.2 *vs* 28.3±1.3 kPa, $P<0.01$). Only a 1.5±1.2 kPa increase in SBP from 16 wk to 40 wk was found in SHR_{cap} group (Fig 1).

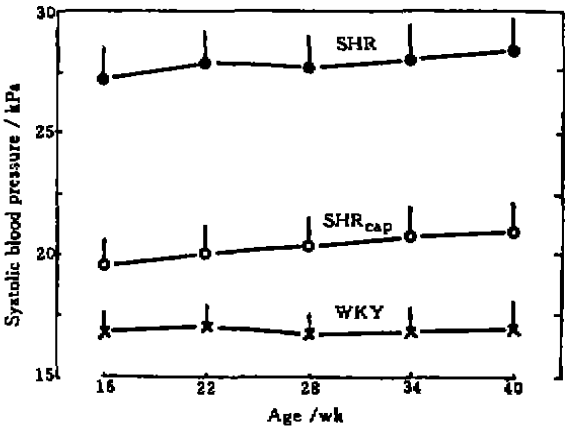

Fig 1. Systolic blood pressure of treated ($n=28$) and untreated ($n=28$) SHR and WKY ($n=28$) from 16 until 40 wk of age. Captopril treatment (100 mg ·kg^{-1}·d^{-1}) was removed in SHR_{cap} at 16 wk of age.

No significant changes were seen in HR among the 3 rat groups. However, at 40 wk of age, the BW of WKY was significantly greater than that of untreated and treated SHR. Cap therapy did not affect BW. LVH

was found in untreated SHR *vs* WKY ($P<0.01$). LVW/BW was markedly reduced in the treated group and almost reversed the level of WKY rats (Tab 1).

Tab 1. Characteristics of treated and untreated spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) at 40 wk of age. $^bP<0.05$ as compared with SHR, $^cP<0.05$ as compared with WKY.

	n	SHR	SHR_{cap}	WKY
Heart rate/bpm	28	358±33	359±32	356±28
Body wt/g	28	333±20^c	326±21^c	380±41^b
LVW/BW/g·kg^{-1}	22	3.58±0.2	2.56±0.18^b	2.33±0.2^b

Northern hybridization demonstrated that both the c-*myc* and c-*fos* probes reacted with single RNA species in preparation of total RNA from LV of the 3 groups of rats (Fig 2), two known sizes being 2.4 kb for c-*myc* mRNA and 2.2 kb for c-*fos* mRNA. LV sample from SHR expressed 3.8-fold ($P<0.01$) higher c-*myc* mRNA/18S rRNA ratio, in comparison with that from WKY rats (Fig 2). There was a decrease in c-*myc* mRNA in the LV sample in SHR_{cap} compared with that in SHR (0.57 ± 0.13 *vs* 2.07 ± 0.16, c-*myc* mRNA/18S rRNA ratio, arbitrary densitometric units, $P<0.01$). Quantification of the extent of the hybridization showed no significant difference in the c-*myc* mRNA levels between LV sample from WKY and SHR_{cap}. Compared with c-*myc* expression, c-*fos* expression was not different in LV in the three groups of rats. The magnitude of LV c-*fos* mRNA in 40-week-old SHR was similar to that in age-matched WKY (1.01 ± 0.16 *vs* 1.01 ± 0.17 c-*fos* mRNA/18S rRNA, P = NS). Although early Cap treatment prevented LVH in SHR, visual inspection of the autoradiographs indicated that Cap therapy did not affect c-*fos* expression of myocyte. No substantially different expression of c-*fos* was found between LV in SHR_{cap} and in SHR of the same age (Tab 2).

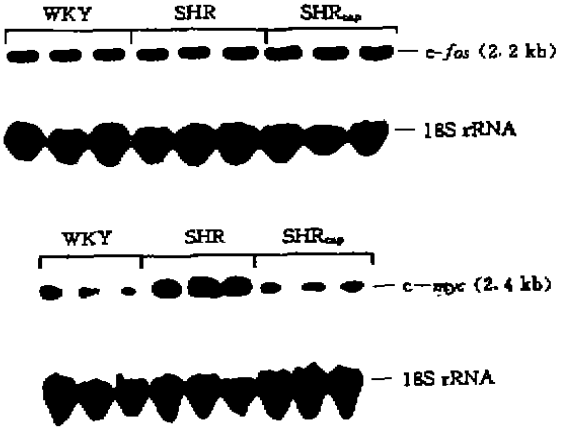

Fig 2. Northern blot showing the levels of protooncogenes c-*myc* and c-*fos* mRNA in LV in SHR, SHR_{cap}, WKY at 40 wk of age. Rehybridization of the same blot with 18S oligonucletide probe indicated that equal amounts of RNA were loaded in each lane (bottom). Similar results were obtained from 2 and 3 additional experimnts, respectively.

Tab 2. C-*myc* and c-*fos* transcript levels corrected for 18S rRNA level. $^bP<0.05$ *vs* SHR.

mRNA/18S rRNA	*n*	SHR	SHR_{cap}	WKY
c-*myc*	5	2.07±0.16	0.57±0.13^b	0.54±0.11^b
c-*fos*	7	1.01±0.16	1.05±0.15	1.01±0.17

DISCUSSION

It has been demonstrated that during the development of genetic hypertension there is a critical phase of sensitivity to pharmaceutical interference[6]. Recent study by Wu and Berecek[7] showed that intrauterine treatment with Cap prevented the development of hypertension in SHR, the effect even being maintained after cessation of therapy. Our results corroborated their observation. Although the

exact mechanism resulting in the prolonged hypotensive effect following Cap therapy remains obscure, ours (data not published) and other data showed the Cap's ability to block AII-induced vascular contraction and hypertrophy which may amplify vasoconstriction[7] and its ability to suppress degradation of bradykinin and kinin which is a potent activator of nitric oxide (NO) endothelium-derived relaxing factor (EDRF), a powerful vasodilator[8], both the properties of Cap contributing greatly to preventing BP elevation for a long time.

The proto-oncogene *c-myc* encodes transcriptional factor and regulates celluar proliferation and differentiation. There was evidence that the expression of *c-myc* gene was detected in the heart of both neonatal and adult rats, and its expression gradually decreased after birth[9]. Furthermore, an increase in the cell size of nondividing cardiac myocytes induced by A Ⅱ, norepinephrine (NE) was preceded by rapid and transient increased expression of *c-myc* gene. Cardiac hypertrophy induced by mechanical overload *in vivo* also exhibited similar results. Excess *c-myc* expression in transgenic animals also induced an increase in heart size[10]. Our results showed that the expression of *c-myc* gene was markedly increased in LV of SHR at 40 wk of age compared with that of age-matched WKY. These results demonstrated that the proto-oncogene *c-myc* expression may play a significant role in the development of hypertrophy. Recently, Shi *et al*[11] showed that antisense *c-myc* oligomers exhibited a significant antiproliferative effect on smooth muscle cells *in vitro* and *in vivo*. Their findings support a role for *c-myc* gene product in mediating a hypertrophic response. Several data *in vivo* showed that AII plays a critical role in pathophysiology and maintenance of LVH[12]. ACEI was sufficient to reduce LVH in SHR, concomitant administration of AII with ACEI prevented the effect of ACEI[13]. Another data showed that treatment with ACEI in SHR lowered LVW and LV AII content, and a significantly positive correlation between them[14]. Even more intriguing were our results, which revealed a marked decrease in *c-myc* mRNA in SHR_{cap} with reversed LVH compared with that in SHR, and also showed the *c-myc* mRNA expression levels in SHR_{cap} to be almost the same as those in WKY. Inhibition of LVH by Cap therapy may result from the attenuation of local AII content, and AII-induced sympathetic drive, which result in supression of *c-myc* expression and subsequently preventing the fetal contractile protein synthesis. The proto-oncogene *c-fos* also encodes nuclear protein and control cellular proliferation and differentiation. In contrast to *c-myc*, in the intact animal *c-fos* expression in cardiac muscle gradually increased with age[9]. Like *c-myc* gene, in acute experiments, a variety of humoral or mechanical stimuli inducing cardiac hypertrophy *in vitro* and *in vivo* elicit rapid and transient expression of *c-fos* gene preceding expression of fetal contractile protein gene. However, it is still unknown whether *c-fos* gene stimulation is essential for the development of ventricular hypertrophy. Our study showed that levels of *c-fos* mRNA were not appreciably increased in intact LV of 40-wk-old SHR compared with that of age-matched WKY. Interestingly, chronic administration of Cap did not change the expression of *c-fos* gene even if LVH had been inhibited. It seems that no *c-fos* expression may be needed for development of cardiac hypertrophy *in vivo* although we can not explain the phenomenon in detail.

Clinical Implications: Our study demonstrated that enhanced expression of *c-myc* is essential for the development of LVH. LVH

inhibition may be mediated by decreased c-*myc* expression. It is anticipated that the c-*myc* antisene oligomers may have therapeutic role in the reversal of LVH.

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卡托普利早期治疗自发性高血压大鼠抑制左室肥厚和 c-*myc* 表达而不影响 c-*fos* 表达

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A **目的**：探讨早期卡托普利治疗抑制左室肥厚的机制。**方法**：♀ SHR 宫内期给药（100 mg·kg^{-1}·d^{-1}）到16周、40周处死，测定收缩压，左室重与体重比，左室 c-*myc* 和 c-*fos* 表达量（Northern 杂交）。**结果**：治疗明显降低血压，停药后24周，仍维持较低血压（20.9±1.2 *vs* 对照 SHR 28.3±1.3 kPa，$P<0.01$）并抑制左室肥厚，心肌 c-*myc* 表达明显减少（0.57±0.13 *vs* 对照 SHR 2.07±0.16，c-*myc* mRNA/18S rRNA，$P<0.01$），c-*fos* 表达无变化。**结论**：卡托普利持久地阻止高血压形成，抑制左室肥厚。后者可能是抑制 c-*myc* 表达结果，治疗不改变 c-*fos* 表达。

关键词 左心室肥厚；原癌基因蛋白 c-*myc*；原癌基因蛋白 c-*fos*；卡托普利；近交 SHR 大鼠