rat myocytes during metabolic inhibition. R 965.1
J Mol Cell Cardiol 1992; 24: 435 - 46 R 972
233-235

卡托普利和依那普利拉对分离的大鼠心肌细胞内游离 Ca^{2+} 浓度的影响[1]

王 军，章 鲁[2]，齐建华，张佩瑾，魏丕敬，顾培坤[3]，全正均 （上海第二医科大学药理教研室，[2]医用计算机教研室，[3]电生理教研室，上海 200025，中国）

关键词 Fura-2；近交 SHR 大鼠；近交 WKY 大鼠；心肌；维拉帕米；卡托普利；依那普利拉

目的：研究转换酶抑制剂卡托普利（Cap）和依那普利拉（Ena）对 SHR 和 WKY 大鼠心肌细胞内游离 Ca^{2+} 浓度的影响 **方法**：用荧光探针 Fura 2-AM 结合计算机图象处理技术测定分离心肌细胞内游离 Ca^{2+} 浓度 **结果**：SHR 心肌细胞内游离 Ca^{2+} 浓度（174 ± 5 nmol·L^{-1}）较 WKY 大鼠（148 ± 15 nmol·L^{-1}）高（$P < 0.01$） Cap 和 Ena 能明显降低 SHR 心肌细胞内游离 Ca^{2+} 浓度（分别为 161 ± 11 和 166 ± 7 nmol·L^{-1}，P 分别 < 0.05），但对 WKY 的无影响（$P > 0.05$）。两药均能明显降低 NE 和 Ang II 引起的 SHR 和 WKY 大鼠心肌细胞内 Ca^{2+} 升高，同时也能明显降低 KCl 引起的 SHR 细胞内 Ca^{2+} 升高（$P < 0.05$），但对 WKY 大鼠的无明显影响（$P > 0.05$） **结论**：Cap 和 Ena 对病理性电压依赖性 Ca^{2+} 通道有直接抑制作用。

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 May; 17 (3): 235 - 238

Effects of menidipine on dihydropyridines binding sites in cardiac and cerebral membranes from old rats with left ventricular hypertrophy[1]

XI Tao[2], RAO Man-Ren
(*Department of Cardiovascular Pharmacology, Nanjing Medical University, Nanjing 210029, China*)

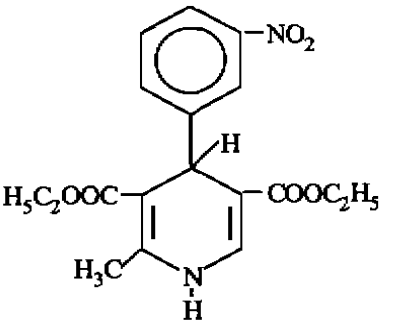
KEY WORDS menidipine; left ventricular hypertrophy; renovascular hypertension; heart; radioligand assay; brain; cell membrane

AIM: To study the effects of menidipine (Men) on the affinity and density of dihydropyridines (DHP) binding sites in the cell membranes of left ventricle (LV) and brain in elderly renovascular hypertensive rats (RVHR) with LV hypertrophy. **METHODS**: Renovascular hypertension was produced by clipping the left renal artery in 20-month-old rats. The affinity and density of DHP binding sites in the cell membranes of LV and brain were assessed by radioligand assay. **RESULTS**: Men (20 mg·kg^{-1}·d^{-1} ig for 9 wk) decreased markedly the systolic blood pressure and the LV weight ($P < 0.01$). Though not affecting the density of DHP receptor (B_{max}), Men markedly decreased the total number of DHP binding sites in hypertrophied LV (from 5.95 ± 0.62 to 4.0 ± 1.1 pmol·LV). Men also reduced B_{max} of DHP binding sites in the thalamus (from 522 ± 27 to 371 ± 24 pmol/g protein) and hippocampus (from 498 ± 26 to 332 ± 32 pmol/g protein). **CONCLUSION**: Men reversed the LV hypertrophy from renovascular hypertension accompanied with reduced total number of DHP binding sites in the cell membranes of LV and B_{max} of the cell membranes of thalamus and hippocampus from elderly LV hypertrophied rats.

Menidipine (Men) is a new calcium antagonist of dihydropyridines (DHP) exploited by our Laboratory. The effects of Men in improving the left ventricular pump function, MVO_2, CO_2 production, in reducing the peripheral vascular resistance and in increasing the coronary flow were stronger and lasting longer than those of nifedipine

[1] Project supported by the National Natural Science Foundation of China, № 38970838.
[2] Now in *Department of Biochemistry, Nanjing University, Nanjing* 210008, *China*.
Received 1995-01-24 Accepted 1995-12-04

(Nif)[1,2]. Men protected the myocardium from global ischemia and reperfusion and isoproterenol-induced injury by inhibiting the transsarcolemmal calcium influx[3]. Since left ventricular hypertrophy (LVH) may be reversed with calcium antagonists in experimental and clinical hypertension[4,5], we tried to evaluate the effects of prolonged treatment with Men on the cardiac mass and calcium antagonist binding sites in the heart and brain of 2K-1C Goldblatt elderly hypertensive rats.



Menidipine

MATERIALS AND METHODS

Reagents Men was obtained from Institute of Tianjin Pharmaceutical Industry. (±) Isradipine ($PN_{200-110}$) was from Sandoz Co, Switzerland. (+)[^{3}H]Isradipine (specific activety 3219 TBq·mol^{-1}) was purchased from Amersham International, UK. All other reagents were AR grade and were prepared using distilled water.

Rat model Sixteen-month-old ♂ Sprague-Dawley rats weighing 370 ± s 30 g (n = 18) were used. Systolic blood pressure (SBP) was measured weekly under conscious and warm (37 ℃) conditions by tail-cuff method using a BP recorder for rats (Shanghai). Two kidney-one clip (2K-1C) hypertension was produced in rats by placing a silver clip (0.3 mm gap) around the left renal artery under anesthesia. Rats were considered hypertensive only if SBP exceeded 20 kPa (150 mmHg) during a 4-wk follow-up period.

Since there is a tendency toward impaired LV performance and LVH in rats with renovascular hypertension of 4-wk duration as compared with sham-operated rats[5], apparent functional deficit and LVH (ratio of LV weight/body weight increased significantly) were even more pronounced in rats with untreated hypertension in an 8-wk duration.

The elderly rats were randomly divided into 3 groups (6/group): A) Sham operated: normotensive rats treated with normal saline 1 mL·kg^{-1}·d^{-1} ig for 9 wk; B) LVH: untreated hypertensive rats with saline 1 mL·kg^{-1}·d^{-1}; C) Men: LVH and treated 9 wk postclipping with Men 20 mg·kg^{-1}·d^{-1} ig for 9 wk.

Radioligand assay The rats were killed by cervical dislocation. The heart, cerebral cortex, thalamus, and hippocampus were immediately placed in a cold homogenizing medium containing $NaHCO_3$ 20 mmol·L^{-1} and phenylmethylsulfonyl fluoride (PMSF) 0.1 mmol·L^{-1}. Cardiac and brain cell membranes were isolated[7]. The pellets from the final spin were resuspended in Tris-HCl buffer solution 50 mmol·L^{-1}.

(+)[^{3}H]Isradipine binding was performed in duplicate using a protein concentration of 0.25 – 0.5 g·L^{-1} in a final volume of 0.25 mL (pH 7.4). For saturation binding (+)[^{3}H]isradipine 0.015 – 1 nmol·L^{-1} was used. Nonspecific binding was measured in the presence of unlabeled (+)isradipine 1 μmol·L^{-1}. Incubation was carried out in the dark for 60 min at 37 ℃. The bound and free (+)[^{3}H]isradipine were separated by rapid filtration through a glass fiber Hong-Guang Type-69 under vacuum. The filter was washed 3 times with Tris-HCl buffer 50 mmol·L^{-1}. The radioactivety of the filters was assayed in a liquid scintillation counter (40 % efficiency, Packard, Tricarb 2200 CA, USA).

Statistics All data were expressed as $\bar{x} \pm s$. Statistical significance of differences was evaluated by t test.

RESULTS

SBP and LV weight Clipping of the left renal artery induced a pronounced hypertension in rats, whereas blood pressure in Men-treated rats was approximately normal (Tab 1).

Tab 1. Effects of Men on blood pressure in 2K-1C hypertensive rats.
n = 6, $\bar{x} \pm s$. $^cP < 0.01$ *vs* before. $^tP < 0.01$ *vs* Sham.

	Before Men/kPa	After Men/kPa
Sham operated	13.78 ± 0.67	13.47 ± 0.88
LVH	24.98 ± 2.87^t	25.78 ± 1.93^t
Men	25.16 ± 1.80^t	15.85 ± 1.38^c

In renovascular hypertensive rats (RVHR), the LV was heavier than that in the sham clipped rats, indicating a real LVH. Men markedly reduced the LV weight in RVHR *vs* untreated rats (Tab 2).

[^{3}H]Isradipine binding to LV and brain membranes [^{3}H]Isradipine bound to the membranes of LV, cortex, thalamus, and hippocampus in a saturable manner and with high affinity to a single

Tab 2. Effects of Men of Left ventricular weight (LVW) and body weight (BW) in 2K-1C hypertensive rats. $n = 6$, $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* LVH; $^dP > 0.05$, $^fP < 0.01$ *vs* Sham.

	Sham operated	LVH	Men
BW/g	478 ± 34	458 ± 62[a]	256 ± 57[d]
LVW/mg	596 ± 77	1 030 ± 61[c]	700 ± 61[f]
LVW:BW/mg·g^{-1}	1.25 ± 0.17	2.29 ± 0.37[c]	1.55 ± 0.17[f]

binding site. In the membranes of LVH rats, K_D ualues were not significantly elevated as compared with those in the sham operated rats. There were noticeable differences in the B_{max} of LV, cortex, thalamus, and hippocampus membranes between LVH and those in the sham operated rats. There was a difference in the total number of receptors per LV in the LVH hearts *vs* the control ($P < 0.01$) (Tab 3, 4). Hill coefficient was 1.

Tab 3. Effects of Men on (+)[^{3}H]isradipine binding to LVH hearts induced by 2K-1C hypertensive rats. $n = 4$, $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* LVH; $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* Sham.

	B_{max} pmol/g protein	Total number of receptors, pmol/LV	K_D, nmol·L^{-1}
Sham operated	479 ± 19	3.08 ± 0.58	0.26 ± 0.05
LVH	681 ± 77[e]	5.95 ± 0.62[f]	0.26 ± 0.05[d]
Men	589 ± 65[a]	4.0 ± 1.10[c]	0.23 ± 0.05[e]

Tab 4. Effects of Men of (+)[^{3}H]isradipine binding to membranes of cerbral cortex, thalamus, and hippocampus from 2K-1C hypertensive rats. $n = 4$, $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* LVH; $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* Sham.

	Sham operated	LVH	Men
K_D, nmol·L^{-1}			
Cortex	0.20 ± 0.03	0.24 ± 0.07[d]	0.22 ± 0.06[a]
Thalamus	0.10 ± 0.02	0.11 ± 0.02[d]	0.11 ± 0.01[a]
Hippocampus	0.12 ± 0.01	0.12 ± 0.02[d]	0.12 ± 0.01[a]
B_{max}, pmol/g protein			
Cortex	368 ± 27	448 ± 29[e]	394 ± 29[a]
Thalamus	295 ± 16	522 ± 27[f]	371 ± 24[c]
Hippocampus	298 ± 26	498 ± 26[f]	332 ± 32[c]

In the LV membranes, the total number of receptors per LV of Men group were lower than those in the LVH group ($P < 0.01$) (Tab 3).

In the thalamus and hippocampus membranes, the B_{max} values of Men group were lower too (Tab 4).

DISCUSSION

Hypertension in the elderly is commonly accompanied by LVH that is associated with increased morbidity and mortality[8,9]. In its treatment, calcium antagonists display favorable effects[10]. Our present studies indicated that long term treatment with Men might markedly decrease the SBP and regress the LVH induced by chronic pressure overload in elderly renovascular hypertensive rats (RVHR).

To determine whether favorable effects of Men on hypertensive LVH might be related to Ca^{2+} channels, we analyzed the changes of LV and various areas of brain membranes from elderly LVH rats by treating with Men. Results of these studies demonstrated that the density and total number of DHP binding sites per LV was significantly increased in the elderly LVH rats. This indicated that the LVH induced by pressure overload was associated with an increased density of DHP receptors on the sarcolemma.

Anyersa *et al*[11] reported that the surface/volume ratio of hypertrophied myocytes remained constant. The density of calcium channels was maintained in the hypertrophied heart by an increased syntheses of channels, particularly in the T-tubular system. The number of functional channels was also increased by pressure overload thus allowing an increase in Ca^{2+} influx necessary for maintaining the contraction of hypertrophied myocytes. Men could significantly decrease the total number of DHP binding sites in the hypertrophied LV induced by renovascular hypertension, which might be through its effects of decreased pressure and reduced ventricular weight. Since the binding sites are associated with the L-type-sensitive Ca^{2+} channels, treatment with calcium antagonists such as Men may prevent the occurrence of intracellular Ca^{2+} overload through inhibiting the transsarcolemmal Ca^{2+} influx in

elderly LVH rats.

The present investigation involving various areas of the brain demonstrated that the numbers of DHP binding sites in the cortex, thalamus, and hippocampus in elderly RVHR increased more than those in elderly sham operated rats. The above results were in accordance with those[12]. We considered that the increased number of DHP binding sites in the cortex, thalamus, and hippocampus of elderly RVHR reflected an increase in DHP calcium channels similar to that in muscle. However, the areas of the brain which might be related to the elevated SBP could not be clearly specified.

In conclusion, Men reduced significantly the SBP and LV mass in hypertensive LVH which are accompanied by reduced number of DHP binding sites in the LV, cortex, thalamus, and hippocampus from elderly RVHR. Men may prevent the occurrence of intracellular Ca^{2+} overload through voltage-sensitive calcium channels. These results indicated that Men displayed favorable effects on hypertensive LVH from elderly rats.

REFERENCES

1 Rao MR, Liang MD, Liu GY, Liu F, Zhang HQ. Effect of *m*-nifedipine, a calcium antagonist, on cardiac performance and oxygen consumption in anesthetized animal: a comparison with nifedipine. Acta Pharm Sin 1984; **19**: 101 - 7.

2 Rao MR, Liang MD, Liu F, Shen XH, Zou X
Effects of *m*-nifedipine on contractile responses in the isolated atria and coronary vassels: a comparison with nifedipine.
Acta Pharm Sin 1986; **21**: 321 - 6.

3 Chen NH, Rao MR. Protective effects of *m*-nifedipine and nifedipine on ischemic-reperfused injury in working guinea pig hearts. Acta Pharmacol Sin 1989; **10**: 156 - 61.

4 Buser PT, Wagner S, Wu ST, Derugin N, Parmley WW, Higgins CB, *et al*. Verapamil preserves myocardial performance and energy metabolism in left ventricular hypertrophy following ischemia and reperfusion. Circulation 1989; **80**: 1837 - 45.

5 Modena MG, Mattioli AV, Parato VM, Mattioli G. Effect of antihypertensive treatment with nitrendipine on left ventricular mass and diastolic filling in patients with mild to moderate hypertension. J Cardiovasc Pharmacol 1992; **19**: 148 - 53.

6 Kuwajima I, Kardon MB, Pegram BL, Sesoko S, Frohlich ED. Regression of left ventricular hypertrophy in two kidney, one clip Goldblatt hypertension
Hypertension 1982; **4** (3 Pt 2): 113 - 8.

7 Glossmann H, Ferry DR. Assay for calcium channels.
Methods Enzymol 1985; **109**: 513 - 50.

8 Kannel WB. Prevalence and natural history of electrocardiographic left ventricular hypertrophy
Am J Med 1983 Sep 26; **75** (3A): 4 - 11.

9 Kannel WB. Role of blood pressure in cardiovascular morbidity and mortality. Prog Cardiovasc Dis 1974; **17**: 5 - 24.

10 Hachamovitch R, Sommenblick EH, Strom JA, Frishman WH Left ventricular hypertrophy in hypertension and the effects of antihypertensive drug therapy.
Curr Probl Cardiol 1988; **13**: 371 - 421.

11 Anversa P, Loud AV, Giacomelli F. Absolute morphometric study of myocardial hypertrophy in experimental hypertension Lab Invest 1978; **38**: 597 - 605.

12 Ishii K, Kano J, Ando J, Yoshida H. Binding of [^{3}H]nitrendipine to cardiac and cerebral membranes from normotensive and renal, deoxycorticosterone/NaCl and spontaneously hypertensive rats. Eur J Pharmacol 1986; **123**: 271 - 8.

235-238

间硝苯啶对老龄左室肥厚大鼠心肌及脑细胞膜二氢吡啶类受体的影响

R966

R972

奚 涛，饶曼人
(南京医科大学心血管药理研究室，南京 210029，中国)

关键词 间硝苯啶；左室肥厚；肾血管高血压；心脏；放射配位体测定；脑；细胞膜

A **目的**：研究间硝苯啶(Men)对老龄左室肥厚大鼠心肌及脑细胞膜二氢吡啶类(DHP)受体的影响. **方法**：选用 20 月龄大鼠，钳夹其左肾动脉形成肾血管高血压. 采用放射配基分析法，测大鼠心肌及脑细胞膜 DHP 结合位点的亲和力和密度(B_{max}). **结果**：Men 20 mg·kg^{-1}·d^{-1} ig 持续 9 周，使老龄肾血管高血压大鼠收缩压及左室重显著降低，减低左室 DHP 受体总量(从 5 95±0 62 降到 4 0±1 1 pmol/LV) Men 也降低丘脑和海马 DHP 受体 B_{max}(分别从 522±27 降到 371±24 pmol/g protein 和从 498±26 降到 332±32 pmol/g protein). **结论**：Men 在逆转老龄肾血管高血压 LVH 同时伴有左室，丘脑和海马钙受体数的降低.