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Effects of *m*-nifedipine on dihydropyridine binding sites in cardiac and cerebral cortex cell membranes from left ventricular hypertrophied rats[1]

XI Tao, RAO Man-Ren (*Department of Cardiovascular Pharmacology, Nanjing Medical College, Nanjing 210029, China*)

ABSTRACT *m*-Nifedipine (*m*-Nif 20 mg·kg^{-1}·d^{-1} ig for 9 wk) decreased left ventricular weight in the renovascular hypertensive rats ($P<0.01$). Though not significantly affecting the density of dihydropyridines (DHP) receptor (B_{max}), *m*-Nif administered whether for prevention (6 wk postclipping) or for regression (9 wk postclipping), markedly decreased the total number of DHP binding sites in hypertrophied left ventricle (LV). *m*-Nif also reduced the dissociation constant (K_d) of DHP binding sites in the membranes of LV and cerebral cortex from cardiac hypertrophied rats ($P<0.01$). These effects of *m*-Nif were similar to those of nifedipine (Nif) in the same dosage. The results suggest that *m*-Nif can prevent and regress the LV hypertrophy resulted from renovascular hypertension and reduce the total number of DHP binding sites in the membranes of LV from cardiac hypertrophied rats.

KEY WORDS *m*-nifedipine; nifedipine; hypertrophy; renovascular hypertension; heart ventricle; dihydropyridines; receptors

Left ventricular hypertrophy (LVH) induced by pressure overload is associated with increased morbidity and mortality of cardiovascular diseases. LVH may be regressed with calcium antagonists in experimental or clinical hypertension[1-5].

Our previous studies suggested that the effects of *m*-nifedipine (*m*-Nif) 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 longer-lasting than those of Nif[6,7]. *m*-Nif protected the myocardium from global ischemia and reperfusion and isoproterenol-induced injury by inhibiting the transsarcolemmal calcium influx[8].

Thus, we try to evaluate the effects of prolonged treatment with *m*-Nif on the cardiac mass and calcium antagonist binding sites in the heart and cerebral cortex of 2K-1C Goldblatt hypertensive rats. Nif was used as a control.

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MATERIALS AND METHODS

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

Rat model Adult ♂ Sprague-Dawley rats, weighing 270±*s* 42 g ($n=48$), were used. They were maintained on a standard diet. Systolic blood pressure (SBP) was measured weekly under conscious, and warm conditions by tail-cuff method using a BP recorder for rats (MRS-III). Hypertension (2K-1C) was brought about in rats by placing a silver clip (0.3 mm gap); around the left renal artery under anesthesia, without touching the right kidney. The sham-operated rats underwent a similar procedure except that the renal artery was not clipped. After surgery they were housed in polyethylene cages with standard rat chow and water *ad lib*.

Rats were considered hypertensive when SBP exceeded 20 kPa (150 mm Hg) during a 4 wk follow-up period.

Since Kuwajima *et al* reported a tendency toward impaired LV performance and LVH in rats with reno-

vascular hypertension of 4-wk duration as compared with sham-operated rats[9], 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 rats were randomly divided into 6 groups (8/group): A) Sham-operated group: normotensive rats treated with same volume of solvent as that of *m*-Nif_2; B) LVH group: non-treated hypertensive rats with same volume of normal saline; C) *m*-Nif_1-treated group: LVH prevented by *m*-Nif, 6 wk postclipping *m*-Nif 20 mg·kg^{-1}·d^{-1} ig for 9 wk; D) Nif_1-treated group: LVH prevented by Nif, procedures and doses were the same as those of *m*-Nif_1; E) *m*-Nif_2-treated group: LVH regressed by *m*-Nif, 9 wk postclipping *m*-Nif 20 mg·kg^{-1}·d^{-1} ig for 9 wk; F) Nif_2-treated group: LVH regressed by Nif, procedures and doses were the same as those of *m*-Nif_2.

Tissue preparation for binding assay The rats were killed by cervical dislocation. After LV was weighed, LV and cerebral cortex 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[10]. The pellets from the final spin were resuspended in Tris-HCl buffer solution 50 mmol·L^{-1} to obtain a final protein concentration of 0.25—0.5 mg·ml^{-1} for binding assay.

Radioligand binding studies (+)[^{3}H]Isradipine binding was performed in duplicate using a protein concentration of 0.25—0.5 mg·ml^{-1} in a final volume of 0.25 ml. The incubation buffer contained Tris-HCl 50 mmol·L^{-1} and PMSF 0.1 mmol·L^{-1} (pH 7.4). For saturation binding (+)[^{3}H]isradipine 0.015—1 nmol·L^{-1} was used. Nonspecific binding was defined by adding (±) isradipine 1 μmol·L^{-1}. Incubation was carried out in the dark for 60 min at 37℃. After incubation, 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 time with Tris-HCl buffer 50 mmol·L^{-1}. The radioactivity of the filters was assayed (40 % efficiency) in a liquid scintillation counter (Packard, Tricarb 2200 CA, USA). The non-specific binding represented about 20—30 % of the total binding.

Statistics Statistical significances of differences between $\bar{x}\pm s$ were evaluated by *t* test.

RESULTS

SBP and LV weight Clipping of the left renal artery induced a pronounced hypertension in rats. In the groups of *m*-Nif_1, Nif_1, *m*-Nif_2, and Nif_2, the SBP was decreased by 47±12%, 46±10%, 27±6%, and 42±7%, respectively. In the renovascular hypertensive rats (RVHR), the LV was heavier than that in the sham clipped rats, suggesting that LVH really existed in RVHR. In the groups of *m*-Nif_1, and *m*-Nif_2, *m*-Nif markedly reduced the LV weight in RVHR *vs* untreated rats. This showed that *m*-Nif could mitigate LVH. The effects of the same dose of Nif on LV weight were similar to those of *m*-Nif (Tab 1).

Tab 1. Effects of *m*-nifedipine and nifedipine on SBP and LVW in renovascular hypertensive rats. $n=6$. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.01$ *vs* before. $^dP>0.05$, $^fP<0.01$ *vs* LVH.

Groups	Systolic blood pressure/kPa Before	After	Left ventricular weight/g
Sham operation	13.67±0.51^f	13.43±0.88^a	0.50±0.05^f
LVH	24.18±2.87	24.97±1.93^a	0.86±0.12
m-Nif_1	25.38±1.64^d	13.42±0.85^c	0.54±0.05^f
Nif_1	25.28±2.40^d	13.65±0.65^c	0.55±0.06^f
m-Nif_2	24.48±1.60^d	17.77±2.87^c	0.54±0.06^f
Nif_2	23.27±2.90^d	13.40±0.85^c	0.58±0.06^f

[^{3}H]Isradipine binding to LV and cerebral cortex membranes [^{3}H]Isradipine binds to the LV and cortex membranes tested in a saturable manner and with high affinity to a single binding site. In the LV and cortex membranes of LVH rats, K_d values were elevated (Tab 2, 3), as compared with those in the sham operated rats. There were no noticeable difference in the B_{max} of LV and cortex

membranes between LVH and sham operated groups, but there was a difference in the total number of DHP receptors expressed per LV in the hypertrophied hearts *vs* the control ($P<0.05$). Hill coefficient calculated was 0.99.

Tab 2. Effects of *m*-nifedipine and nifedipine on DHP binding sites in LV membranes from LV hypertrophied hearts of 2k-1c hypertensive rats. $n=4$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* LVH.

Groups	K_d pmol·L^{-1}	B_{max} fmol/mg protein	Total number of receptors pmol/LV
Sham operation	211±18^c	704±50^a	5.3±0.4^c
m-Nif$_1$	200±40^c	729±84^a	5.8±0.7^b
Nif$_1$	217±25^c	781±93^a	5.7±0.8^b
m-Nif$_2$	200±30^c	688±25^a	4.7±0.5^c
Nif$_2$	210±50^c	686±49^a	4.9±0.6^c
LVH	382±22	786±81	7.2±0.6

Tab 3. Effects of *m*-nifedipine and nifedipine on DHP binding sites in cerebral cortex membranes from 2k-1c hypertensive rats. $n=4$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* LVH.

Groups	K_d pmol·L^{-1}	B_{max} fmol/mg protein
Sham operation	238±25^b	666±64^a
m-Nif$_1$	206±12^c	675±74^a
Nif$_1$	228±17^c	755±72^a
m-Nif$_2$	207±25^c	626±80^a
Nif$_2$	203±20^c	654±14^a
LVH	320±30	683±74

Effects of *m*-Nif and Nif on [^{3}H]isradipine binding In the LV membranes, the total number of DHP receptors per LV in the *m*-Nif$_1$, Nif$_1$, *m*-Nif$_2$, and Nif$_2$ groups were lower than those in the LVH group ($P<0.05$ and $P<0.01$). The K_d values in the these groups were also much lower ($P<0.01$). There were no marked differences between the effects of *m*-Nif and Nif (Tab 2). In the cortex membrane, the K_d values in these groups were significantly lower too (Tab 3).

DISCUSSION

The data obtained in this study confirmed that long term treatment with *m*-Nif may markedly decrease the blood pressure and prevent or regress LVH induced by chronic pressure overload in renovascular hypertensive rats, similar to those treated with Nif.

The major aim of the present study was to utilize a ligand binding study of [^{3}H]isradipine to analyse the changes of the affinity and density of dihydropyridine binding sites in the LV and cerebral cortex membranes during the onset of hypertrophy in rat hearts and to investigate the biochemical features whereby *m*-Nif and Nif could prevent and regress the LVH. The results of these studies demonstrated that the total number of DHP binding sites per LV was significantly increased in the LVH rats. This may be related either to an increased density of receptors on the sarcolemma or to a similar density in an extended area. *m*-Nif and Nif, administered whether for prevention or regression, could significantly decrease the total number of DHP binding sites in the entire hypertrophied LV induced by renovascular hypertension.

According to a morphometric study by Anyersa *et al*[11], the surface/volume ratio of hypertrophied myocytes was remained constant and that the surface of T-tubular system increased approximately 100% in chronically hypertrophied rat hearts. As number of binding sites per milligram of protein was similar and the surface/volume ratio was constant, it was assumed that the density of calcium channels was maintained in the hypertrophied heart by an increased synthesis 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 the hypertrophied myocytes. In a recent study, we also found that *m*-Nif and Nif could markedly decrease the mitochondrial calcium in the hypertrophied myocytes and improve the hypertrophied LV compliance (unpublished data).

In the present study, the K_D value in the heart and cortex of LVH rats was increased. This was likely to be related to a diminution in the Ca^{2+} content of the native membranes. The DHP binding to the heart and cortex is calcium-dependent, indicating that Ca^{2+} channels in both preparations of heart and cortex of LVH rats had calcium domains regulating the DHP binding. Therefore, it may be possible for these calcium domains to modulate the calcium entry through the calcium channels by changing their sensitivity to calcium; and the reduced sensitivity of the calcium channel in hypertensive myocardial hypertrophied hearts may block the protective mechanism against any cytosolic increase in calcium ions.

In conclusion, *m*-Nif and Nif reduced the SBP significantly and prevent and regress the total cardiac and left ventricular mass in hypertensive LVH which are accompanied DHP binding sites regulated. *m*-Nif and Nif both may prevent the occurrence of intracellular Ca^{2+} overload through voltage-sensitive calcium channels. These results indicated that both drugs displayed favorable effects on hypertensive LVH.

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间硝苯地平对左室肥厚大鼠心肌及大脑皮质细胞膜二氢吡啶类受体的影响

姜 涛，饶曼人 R965.2

（南京医学院心血管药理研究室，南京210029，中国）

摘要 间硝苯地平(*m*-Nif)预防性和逆转性给药，可使肾性高血压大鼠肥厚左室二氢吡啶类(DHP)受体总量明显降低. *m*-Nif 也降低左室及大脑皮质 DHP 结合位点的解离常数(K_d). 等量的硝苯地平作用与 *m*-Nif 相似. 提示：*m*-Nif 具有预防和逆转肾性高血压大鼠左室肥厚作用，并可减少肥厚左室 DHP 受体总量.

关键词 间硝苯地平；硝苯地平；肥厚；肾血管高血压；心室；二氢吡啶类；受体

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Effects of hydrochlorothiazide on contraction and ^{86}Rb efflux in rat aorta

WANG Gui-Song, LI Yun-Shan, FU Shao-Xuan
(Department of Pharmacology, Hebei Medical College, Shijiazhuang 050017, China)

ABSTRACT Hydrochlorothiazide (HCT) (0.1, 0.3 mmol·L^{-1}) inhibited the contraction of rat aortic strips induced by low (<40 mmol·L^{-1}), not higher concentrations of KCl. HCT (0.3 mmol·L^{-1}) did not inhibit the $CaCl_2$-induced contraction of the aortic strips depolarized with high K^+ (KCl 80 mmol·L^{-1}). The inhibitory effect of HCT (0.1 mmol·L^{-1}) on KCl (20 mmol·L^{-1})-induced contraction was markedly antagonized by $BaCl_2$ (0.1 mmol·L^{-1}) and tetraethylammonium (TEA) (0.3 mmol·L^{-1}), but not by glibenclamide (Gli, 0.01 mmol·L^{-1}). With norepinephrine (NE) or 5-HT as agonists, HCT (0.3 mmol·L^{-1}) also inhibited the contractions of rat aortic strips. In the 2 components of NE-induced contraction, HCT inhibited only the tonic component depending on Ca^{2+} influx, but not the phasic component elicited by the release of intracellular Ca^{2+}. The inhibitory action of HCT was endothelium-independent. That the HCT (3 mmol·L^{-1}) increased the ^{86}Rb efflux rate coefficient was antagonized by $BaCl_2$ (0.1 mmol·L^{-1}), but not by Gli (0.01 mmol·L^{-1}). The results indicated that the inhibitory effect of HCT on the contraction of rat aorta was attributable to the opening of membrane potassium channels.

KEY WORDS hydrochlorothiazide; barium; tetraethylammonium compounds; glyburide; rubidium; radioisotopes; thoracic aorta

Hydrochlorothiazide (HCT) has long been used in the treatment of hypertension, although its mechanism remains controversial. The central issue of the controversy is whether HCT lowers the blood pressure through volume depletion or by vasodilation[1]. A new class of vasodilators termed 'potassium channel openers' has been identified, and the opening of membrane K^+ channels underlies the vasodilatory effect of diazoxide, a classical antihypertensive agent structurally simillar to HCT[2－5]. In order to determine whether HCT shares with diazoxide a common mechanism of action, we studied the effect of HCT on the mechanical activity and ^{86}Rb efflux in isolated rat aorta.

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MATERIALS AND METHODS

Drugs The Krebs-Henseleit (K-H) solution consisted of NaCl 118, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25, EDTA 0.03, glucose 11 mmol·L^{-1}. In Ca^{2+}-free K-H solution, $CaCl_2$ was precluded. High K^+-depolarized solution was prepared from Ca^{2+}-free solution with KCl 80 mmol·L^{-1}. HCT (Changzhou Pharmaceutical Factory) was dissolved in K-H solution (100 ml) containing *N*,*N*'-dimethylformamide (5 ml), polysorbate 80 (3 ml), and tartaric acid 1 mol·L^{-1} (2 ml) to prepare a stock solution (20 mmol·L^{-1}). Tetraethylammonium chloride (TEA) and $BaCl_2$ (Beijing Chemical