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Effects of tetrandrine on left ventricle hypertrophy in deoxycorticosterone acetate-salt hypertensive rats

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Abstract

The effect of regression of left ventricular hypertrophy was studied in deoxycorticosterone-acetate-salt hypertensive rats (DOCA-salt hypertensive rats) treated with tetrandrine. Treatment with tetrandrine (by gastric intubation, 50 mg/kg per day for 9 weeks) lowered systolic blood pressure, left ventricular weight, Ca^{2+} of mitochondria, and markedly decreased the density (B_{max}) and total number of dihydropyridine binding sites in hypertrophic left ventricle ($P < 0.001$). There was no difference between groups in dissociation constant (K_d) values of dihydropyridine binding sites. These facts indicate that tetrandrine decreased cardiac mass in DOCA-salt hypertensive rats through mechanisms that may be associated with the density and the total number of dihydropyridine binding sites, Ca^{2+} and blood pressure control.

Keywords: Tetrandrine; Hypertrophy; (Receptor); Dihydropyridine; Ca^{2+}; DOCA (deoxycorticosterone acetate)

1. Introduction

Left ventricular hypertrophy is a common complication in hypertension (Cimini and Weiss, 1990). On one hand, development of left ventricular hypertrophy is an adaptive phenomenon that serves as a structural means for the pumping chamber to overcome the hemodynamic afterload imposed on it by the ever-increasing pressure overload of hypertensive vascular disease (Frohlich, 1991). On the other hand, left ventricular hypertrophy confers a very definite risk of increased cardiovascular morbidity and mortality that is independent of the arterial blood pressure (Kannel, 1989). Thus, at the present time the best means for preventing the risk of left ventricular hypertrophy is to prevent its development in the first place (Frohlich, 1991).

Hypertrophy reversal has been achieved in humans and experimental animals with antihypertensive drugs or by removing the causative factors (Sen et al., 1977; Motz and Strauer, 1985). Although the precise mechanisms through which reversal is achieved have not been fully elucidated, they are thought to be mediated in part by both blood pressure reduction and adrenergic blockade (Tarazi et al., 1982). Ca^{2+} channel antagonists are effective antihypertensive agents and recent reports suggest that they may cause reversal of hypertrophy (Motz et al., 1983; Modena et al., 1992) and that their regression of left ventricular hypertrophy is due to their direct effects on the total number of dihydropyridine binding sites (Xi and Rao, 1993).

Tetrandrine is a bis-benzylisoquinoline alkaloid (6,6',7,12-tetramethoxy-2,2'-dimethylberbaman) isolated from Radix Stephania tetrandra S. Moore. It has been clinically used as an antihypertensive and antiarrhythmic agent (Fang and Jiang, 1986) which exerts a negative inotropic effect on the myocardium and blocks high K^+-evoked contraction of arteries, and inhibits the inward Ca^{2+} current in cardiac Purkinje fibers (Wang et al., 1988). Patch-clamp studies have demonstrated that tetrandrine blocks more than one type of voltage-gate Ca^{2+} channel (Liu et al., 1992; King et al., 1988).

However, it is yet unknown how tetrandrine, as an antihypertensive agent, influences the heart weight,

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Ca^{2+} of mitochondria, and dihydropyridine binding sites in the hypertrophic hearts of deoxycorticosterone-acetate-salt hypertensive rats (DOCA-salt hypertensive rats). In an attempt to elucidate this further, we have looked at the effects of tetrandrine on blood pressure, heart weight, Ca^{2+} of mitochondria, and density and affinity of dihydropyridine binding sites in the cardiac hypertrophy of DOCA hypertensive rats.

2. Materials and methods

2.1. Reagents

Tetrandrine was purchased from Jinghua Pharmaceutical Co, China. DOCA was purchased from Sigma Co., USA. (±)-Isradipine was a gift from Sandoz Company, Switzerland. [^{3}H](+)-Isradipine (specific activity 87 Ci/mmol) was purchased from Amersham International, UK. All solutions were prepared using distilled water.

2.2. Rat model

Adult male Sprague-Dawley rats, weighing 280 ± 30 g ($n = 28$), were used. They were maintained on a standard diet. Systolic blood pressure was measured each week in conscious rats using the tail-cuff method (BP recorder for rats, MRS-III, China). The rats had a left nephrectomy under Ketamine anesthesia. DOCA-salt (dissolved in Polysorbate 80, Carmellose-Na, and 1% NaCl) was given subcutaneously (s.c. 5 mg/kg) 7 times weekly for 9 weeks. The rats were fed on a rat chow diet and allowed access to 1 percent saline (1% NaCl) drinking water ad libitum. The normal rats underwent a similar procedure except there was no operation and s.c. DOCA. The rats were considered hypertensive only if systolic pressure exceeded 20 kPa (150 mm Hg) during 4 weeks of the follow-up period. 8 weeks were allowed for cardiac hypertrophy to develop and stabilize. Kuwajima et al. (1982) reported that the apparent functional deficit and left ventricular hypertrophy (left ventricular weight/body weight was increased over 50%) were even greater in rats with untreated hypertension of 8 weeks' duration.

The rats were randomly divided into three groups (each group $n = 8$). (1) Normal group: normotensive rats, treated with the same volume of solvent as that of tetrandrine. (2) Left ventricular hypertrophic group: left ventricular hypertrophic rats treated with the same volume of normal saline. (3) Tetrandrine-treated group: left ventricular hypertrophy reversed by tetrandrine (dissolved in 0.9% NaCl), 50 mg/kg per day by gastric tube for 9 weeks.

2.3. Tissue preparation for binding assay

At the end of the 9-week treatment period, the rats were killed by cervical dislocation. The left ventricle was removed immediately, weighed and placed in cold homogenizing medium containing 20 mM $NaHCO_3$ and 0.1 mM phenylmethylsulfonyl fluoride. Cardiac membranes were isolated using the method described by Glossmann and Ferry (Glossmann and Ferry, 1985). The left ventricle was minced into small pieces, and then homogenized in 10 ml homogenizing medium/g wet weight tissue, using three 10-s bursts of a XHF-1 homogenize (China) at 10000 rotational speed. The resulting homogenates were centrifuged at $1500 \times g$ for 15 min at 4° C in a GL-20A centrifuge (China). The resultant pellet was resuspended and centrifuged again. The supernatants were centrifuged for 15 min at 31000 $\times g$. The resultant pellets were resuspended in ice-cold 50 mM Tris-HCl buffer solution (pH 7.4) and recentrifuged. This procedure was repeated twice. The pellets from the final spin were resuspented in 50 mM Tris-HCl buffer solution to obtain a final protein concentration of 0.25–0.5 mg/ml and were used for the binding assay. Protein concentration was determined by the method of Lowry et al. (Lowry et al., 1951), using bovine serum albumin as standard.

2.4. Radioligand binding studies

[^{3}H](+)-Isradipine binding was monitored as described by Glossmann and Ferry (1985) and was performed in duplicate, using a protein concentration of 0.25–0.5 mg/ml in a final volume of 0.25 ml. The incubation buffer contained 50 mmol/l Tris-HCl and 0.1 mM phenylmethylsulfonyl fluoride (pH 7.4). For saturation binding 0.015–1 nmol/l [^{3}H](+)-isradipine was used. Non-specific binding was defined by adding 10^{-6} mol/l (±)-isradipine. Incubations were carried out in the dark for 60 min at 37° C. After incubation, bound and free [^{3}H](+)-isradipine were separated by rapid filtration through a Hong-Guang of 69-type glass fiber filter (China) under vacuum filtration. The filter was washed 3 times with 8 ml of 50 mmol/l Tris-HCl buffer. After addition of 6 ml of scintillation fluid, the radioactivity of the filters was counted (40% efficiency) in a liquid scintillation counter (Packard, Tricarb 2200, USA). The specific binding represented 20–30% of total binding. Due to the photolability of the dihydropyridines, binding studies were performed under a sodium lamp.

2.5. Mitochondrial Ca^{2+} analysis

Mitochondria were isolated using the general procedures and precautions described by Peng et al. (1977). Myocardium was minced with iris scissors on the lip of

a homogenization tube and then homogenized in 10 ml of homogenizing solution/g of tissue, using 10-s bursts of a XHF-1 homogenizer (China) at 10000 rotational speed for 3 times. The solution contained 0.25 M sucrose and 50 mM Tris buffer (pH 7.4). The homogenate was centrifuged at $225 \times g$. The supernatant was removed and passed through a stainless steel screen, and sedimented at $12100 \times g$ for 10 min. The supernatant was discarded and the pellet was washed with 5 ml of Sucrose-Tris-HCl and resedimented at $12100 \times g$ for 10 min. The pellet (mitochondria) was extracted with 3 ml HNO_3 (1 M) for 24 h at 37° C. The supernatant was diluted with 3 ml HNO_3 (1 M). The Ca^{2+} of mitochondria was analyzed in quadruplicate by flame atomic absorption spectrophotometry in a model AA-1475 (Japan).

2.6. Statistics

All data are expressed as means ± S.D. The statistical significance of differences was examined by means of Student's *t*-test.

3. Results

3.1. Blood pressure control and reversal of hypertrophy

Table 1 illustrates the blood pressure in tetrandrine-treated, left ventricular hypertrophy and normal rats. Before treatment, systolic blood pressure was 26.83 ± 1.05 kPa in left ventricular hypertrophic rats and 13.67 ± 0.51 kPa in normal rats. After treatment, in the left ventricular hypertrophic rats, systolic blood pressure increased from 26.8 ± 1.05 to 27.63 ± 1.43 kPa, while treatment with tetrandrine for 9 weeks significantly decreased systolic blood pressure, the decrease being 45 ± 7%. There was no difference in systolic blood pressure between tetrandrine-treated and normal rats.

Left ventricular free wall wet weight, right ventricular wet weight, ventricular septa wet weight, and body weight in each group are summarized in Table 2. The left ventricular free wall wet weight was significantly heavier in left ventricular hypertrophic rats than in normal rats, whether expressed in absolute values or normalized for body weight. Right ventricular wet weight and ventricular septa wet weight were also different from those of normal rats: left ventricular free wall wet weight was increased by 83%, right ventricular wet weight was increased by 50%, ventricular septa wet weight was increased by 42%; all these changes were statistically significant ($P < 0.001$). 9 weeks' treatment with tetrandrine produced a significant reduction in ventricular weight in both absolute terms and relative to body weight when compared with that of the left ventricular hypertrophic rats. In the tetrandrine-treated rats, the reduction was 41% for left ventricular free wall wet weight, 31% for right ventricular wet weight, and 19% for ventricular septa wet weight. These changes are statistically significant at the 0.1% levels using Students' *t*-test. These results suggest that the order of reversal of hypertrophy by tetrandrine was left ventricle > right ventricle > ventricular septa.

Table 1
Effects of tetrandrine on systolic blood pressure in DOCA-salt hypertensive rats

Groups	Systolic blood pressure (kPa)			
	Control	Before	After	Drop (%)
Normal	14.3 ± 0.9	13.7 ± 0.5	13.4 ± 0.9	1.8 ± 0.2
LVH	13.8 ± 0.6	26.8 ± 0.6 [a,b]	26.4 ± 1.1 [c,b]	4.2 ± 1.7
Tetrandrine	13.9 ± 0.9	27.2 ± 0.6 [a,b]	14.8 ± 0.8 [c,d]	44.6 ± 6.9 [d]

Control: basic blood pressure. Before: blood pressure before drug administration. After: blood pressure after drug administration. Normal: normotensive rats. LVH: left ventricular hypertrophic rats. Tetrandrine: tetrandrine-treated rats. Means ± S.D.; $n = 8$; [a] $P <$ 0.001 compared with Control, [b] $P < 0.001$ compared with Normal, [c] $P < 0.01$ compared with Before, [d] $P < 0.001$ compared with LVH.

Table 2
Effects of tetrandrine on the weight of hypertrophic hearts from DOCA-salt hypertensive rats

	Normal	LVH	Tetrandrine
BW (g)	457.3 ± 27.9	424.4 ± 18.9	443.4 ± 26.7
HWW (mg)	1040.0 ± 48.3	1670.4 ± 55.7 [b]	1114.1 ± 66.9 [a]
LVFWW (mg)	535.9 ± 28.3	982.0 ± 29.1 [b]	563.7 ± 30.6 [a]
VSWW (mg)	257.4 ± 32.5	364.0 ± 42.5 [b]	295.8 ± 38.7 [a]
RVWW (mg)	236.8 ± 21.5	359.4 ± 42.8 [b]	254.4 ± 30.2 [a]
HWW/BW (mg/g)	2.3 ± 0.2	3.9 ± 0.1 [b]	2.5 ± 0.2 [a]
LVFWW/BW (mg/g)	1.2 ± 0.06	2.3 ± 0.1 [b]	1.2 ± 0.1 [a]
VSWW/BW (mg/g)	0.7 ± 0.1	0.9 ± 0.1 [b]	0.7 ± 0.1 [a]
RVWW/BW (mg/g)	0.5 ± 0.1	0.9 ± 0.1 [b]	0.6 ± 0.1 [a]

BW: body weight. HWW: heart wet weight. LVFWW: left ventricular free wall wet weight. RVWW: right ventricular wet weight. VSWW: ventricular septa wet weight. Normal: normotensive rats. LVH: left ventricular hypertrophic rats. Tetrandrine: tetrandrine-treated rats. Means ± S.D.; $n = 8$; [a] $P < 0.001$ compared with LVH, [b] $P < 0.001$ compared with Normal.

3.2. The properties of [^{3}H](+)-isradipine binding to left ventricle from left ventricular hypertrophic and normal rats

Because the Hill coefficient calculated for the experimental conditions was close to 1, [^{3}H](+)-isradipine bound to the left ventricular membranes in a saturated manner and with high affinity to a single binding site. In the left ventricular hypertrophic rats, the B_{max} of left ventricular membranes was significantly increased (Table 3, Fig. 1), as compared with that in the normal rats ($P < 0.01$). There was a significant difference in the total number of dihydropyridine receptors expressed per left ventricle from hypertrophic and normal hearts ($P < 0.001$), but there were no marked

Table 3
Effects of tetrandrine on characteristics of dihydropyridine binding sites in left ventricular hypertrophic membranes from DOCA-salt hypertensive rats

	K_d (nM)	B_{max} (fmol/mg protein)	TNR (pmol/LV)
Normal	0.15 ± 0.01	404.37 ± 17.49	3.92 ± 0.45
LVH	0.17 ± 0.01	835.57 ± 47.42 [b]	9.93 ± 0.21 [b]
Tetrandrine	0.17 ± 0.01	460.63 ± 11.16 [a]	4.55 ± 0.47 [a]

TNR: the total number of receptors. LVH: left ventricular hypertrophic rats. Tetrandrine: tetrandrine-treated rats. Normal: normotensive rats. Means ± S.D.; $n = 8$; [a] $P < 0.001$ compared with LVH, [b] $P < 0.001$ compared with Normal.

differences in the K_d values of left ventricle between the left ventricular hypertrophic and normal rats. This result suggests that, before the development of left ventricular hypertrophy, there were no differences in the density of left ventricular Ca^{2+} channels and their affinity, whereas when left ventricular hypertrophy became established, the density of left ventricular Ca^{2+} channels was greater in left ventricular hypertrophic rats than that in the normal rats (Table 3, Fig. 1).

3.3. Effects of tetrandrine on the properties of [^{3}H](+)-isradipine binding to the left ventricle from left ventricular hypertrophic rats

In the tetrandrine-treated rats, the B_{max} of dihydropyridine in left ventricular membranes was significantly decreased compared with that in the left ventricular hypertrophic rats ($P < 0.001$), and the total number of dihydropyridine receptors per left ventricle of tetrandrine-treated rats was lower than that of left ventricular hypertrophic rats ($P < 0.001$). However, tetrandrine decreased the total number of dihydropyridine receptors per left ventricle by 55%, a significantly greater decrease than that obtained for the B_{max} of dihydropyridine binding sites in left ventricular membranes (45%). There were no marked differences in the K_d values between the tetrandrine-treated, the left ventricular hypertrophic and the normal rats (Table 3, Fig. 1).

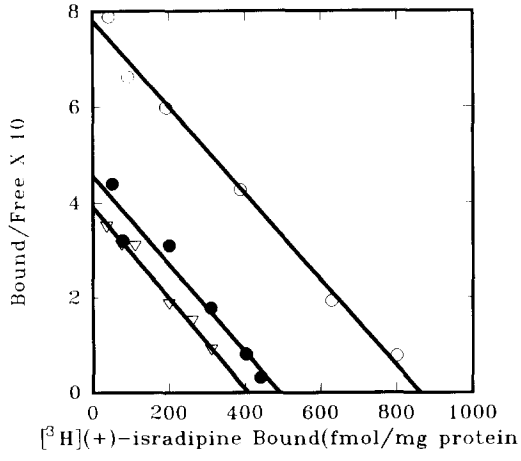

Fig. 1. Scatchard plots of [^{3}H](+)-isradipine binding to left ventricular membranes from hypertrophic hearts from (treated or non-treated) DOCA-salt hypertensive rats. (○): left ventricular hypertrophic rats, (●): tetrandrine-treated rats, (▽): normotensive rats.

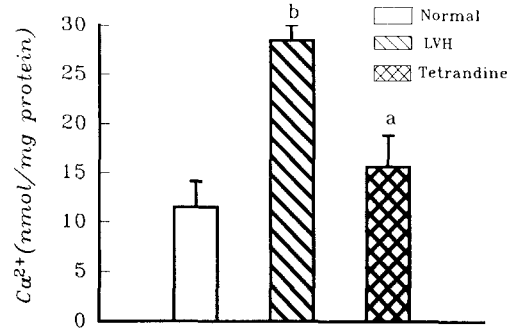

Fig. 2. Effect of tetrandrine on Ca^{2+} content of the cardiac mitochondria in hypertrophic hearts from DOCA-salt hypertensive rats. Normal group: normotensive rats. Tetrandrine group: tetrandrine-treated rats. LVH group: left ventricular hypertrophic rats. Means ± S.D.; $n = 8$; [a] $P < 0.001$ compared with the left ventricular hypertrophic rats; [b] $P < 0.001$ compared with the normal rats.

3.4. Effect of tetrandrine on mitochondrial Ca^{2+} from hypertrophic left ventricle

Basal level of mitochondrial Ca^{2+} in the left ventricle of the normal rats was 11.5 ± 2.6 nmol/mg protein. The mitochondrial Ca^{2+} concentration was significantly higher in the left ventricular hypertrophic rats than in the normal rats, while the mitochondrial Ca^{2+} was significantly lower in the tetrandrine-treated rats than in the left ventricular hypertrophic rats (Fig. 2).

4. Discussion

Tetrandrine is hypotensive in humans (Fang and Jiang, 1986) and protects against chloroform- and adrenaline-induced ventricular fibrillation in cats (Cha et al., 1981). Subsequently, it has been shown to be a Ca^{2+} channel antagonist. In conscious rats intravenous tetrandrine lowers mean systolic and diastolic blood pressure and depresses cardiac contractility for more than 30 min. These effects are consistent with the view that tetrandrine acts as a Ca^{2+} channel antagonist similar to verapamil (Fang and Jiang, 1986), but whole-cell patch-clamp experiments performed on ventricular cells from rat hearts suggest that tetrandrine inhibits both T and L Ca^{2+} channels (Liu et al., 1992). Binding studies using ventricular myocytes and homogenates from rabbit hearts also indicate that tetrandrine interacts at sites associated with Ca^{2+} (King et al., 1988). The results of the present study are consistent with the view that tetrandrine may markedly re-

duce blood pressure and Ca^{2+} of aorta and tail (to be published). The tetrandrine-produced lowering of blood pressure is related to the Ca^{2+} of peripheral arteries. Tetrandrine acts mainly by causing relaxation of vascular smooth muscle, thereby decreasing peripheral resistance and blood pressure.

In the present study we evaluated the effects of chronic treatment with the Ca^{2+} channel antagonist tetrandrine on cardiac anatomy changes resulting in hypertrophic left ventricle. The effects with tetrandrine were assessed by comparing the changes in left ventricular free wall wet weight, right ventricular wet weight and ventricular septa wet weight between the left ventricular hypertrophic rats and the normal rats. The results of this study show that prolonged treatment with tetrandrine may markedly reduce blood pressure and regress left ventricular hypertrophy induced by chronic pressure overload in DOCA hypertensive rats. It suggests that left ventricular hypertrophy is related to the degree of pressure overload, and tetrandrine regressed cardiac mass through mechanisms that may be associated with blood pressure control. Strauer reported that possible pressure-independent factors may help nifedipine in inducing regression of cardiac hypertrophy (Strauer, 1984). Long-term blood pressure control has been associated with regression of left ventricular hypertrophy, but both the development of ventricular hypertrophy in experimental hypertension and its reversal during medical or surgical treatment have been repeatedly shown not to depend solely on hemodynamic factors (Frohlich, 1983, Frohlich, 1991). Moreover, control of arterial pressure may not always result in regression of cardiac mass. Indeed, reversal of cardiac hypertrophy has been demonstrated after surgical cure of experimental hypertension (Kuwajima et al., 1982), and others reported prevention and reversal of hypertrophy in SHR with methyldopa and captopril (Frohlich, 1983). Other antihypertensive agents given in doses having a depressor effect equipotent to that of methyldopa (400 mg/kg per day) either had no effect or even aggravated ventricular hypertrophy (Frohlich, 1991), although arterial pressure and total peripheral resistance were reduced to the same extent or more. These results suggest that one or more factors in addition to pressure reduction could play a significant role in modulating structural ventricular responses to an altered pressure load. Differences in neurohumoral effects could have an important role in the effect of different drugs on cardiac mass, including Ca^{2+} channel antagonists. Drugs that interfere with (or do not activate) the adrenergic and renopressor systems reduce cardiac mass, whereas agents that stimulate these pressor systems seem to have the opposite effect (Frohlich, 1991). However, previous work has shown that prolonged treatment with the slow entry Ca^{2+} channel blocker does not activate the renopressor system (Frohlich, 1991). In general, sympathetic blockade and blood pressure control are also the two independent variables that induce hypertrophy reversal, and their combination is more effective in limiting left ventricular hypertrophy. Ca^{2+} entry blockers are capable of inducing some degree of hypertrophy reversal, but it is not clear whether this is due to their antihypertensive effect or to an intrinsic effect of these agents. These drugs are not sympatholytic since, after long-term administration of Ca^{2+} blockers, circulating catecholamines either increase slightly or do not change (Pedersen et al., 1979).

It is known that Ca^{2+} plays a pivotal role in myocardial contractility and its regulation. In cardiac muscle, sarcoplasmic reticulum Ca^{2+} release requires Ca^{2+} influx rather than simply the charge movement associated with Ca^{2+} channel gating (Nabauer et al., 1989). During the adaptive response of the heart to chronic mechanical overload, qualitative and quantitative changes have been demonstrated in numerous biochemical, metabolic or mechanical studies (Swynghedauw, 1986). Cardiac hypertrophy is usually associated with an alteration in contractility (Schwartz et al., 1981; Lecarpentier et al., 1987), a prolongation of the action potential duration (Nordin et al., 1989), and a diminished heat production, leading to an improved economy of the contractile system. Since Ca^{2+} influx through Ca^{2+} channels is the trigger for Ca^{2+} release from the sarcoplasmic reticulum (Fabiato, 1982), and hence is essential in excitation-contraction coupling, potential changes in dihydropyridine receptors might play an important role in the altered properties of the hypertrophic myocardium. At the sarcolemmal level, the adaptational mechanism to hypertrophy depends on the receptor type. In the hypertrophic rat heart, modifications in both the density and properties of the Na^+/K^+-ATPase have been observed (Charlemagne et al., 1986) while only a decrease in the density of the β-adrenoceptors and muscarinic receptors, without changes in their affinity, has been reported (Chevalier et al., 1989). Another mechanism of adaptation concerns the dihydropyridine receptors, whose total number is increased and whose density is maintained (Ebata et al., 1991; Mayoux et al., 1988). Another important consequence of these findings concerns the biological pathway by which the genes have been activated by the increase in internal Ca^{2+} as an increase in intracellular Ca^{2+} induces oncogene (*c-myc*, *c-fos*) expression (Marban and Koretsune, 1990). Because Ca^{2+} influx is modulated by the voltage-dependent Ca^{2+} channel, the role of Ca^{2+} channel in the maintenance of intracellular Ca^{2+} concentration is very important. Among the Ca^{2+} channel antagonists, dihydropyridine blocks the slow inward current most potently and its receptor is considered to be a putative Ca^{2+} channel. Therefore, a major aim of the present work was to compare

Ca^{2+} channel antagonist binding sites in hypertrophic and normal hearts, and the effect of tetrandrine on dihydropyridine binding sites in hypertrophic left ventricle. This work demonstrated that mitochondrial Ca^{2+} and the density of dihydropyridine binding sites (B_{max}) were increased in rats with myocardial hypertrophy. This can be related either to an increased density of the receptors on the sarcolemma or to a similar density on an increased area. Tetrandrine can significantly decrease B_{max} and the total number of dihydropyridine binding sites in whole, DOCA hypertension-induced hypertrophic left ventricles. These results are consistent with the results of Xi et al. (Xi and Rao, 1993). Others found that the majority of dihydropyridine receptors in cardiac muscle are functional L-type Ca^{2+} channels (Wilbur et al., 1991). Using a variety of biochemical, immunologic and pharmacologic techniques, it has been established that the L-channel probably consists of five subunits, designated, α_1, α_2, β, γ and δ. Bay K8644 increases L-channel Ca^{2+} channel current in a concentration-dependent manner. But dihydropyridine agonists do not affect cAMP levels in heart cells, and the mechanism by which they stimulate $I_{Ca,L}$ is therefore different from that of agents that enhance cAMP-dependent phosphorylation (Mcdonald et al., 1994). The reduction in density of Ca^{2+} channels by tetrandrine might be caused by the blockade of Ca^{2+} channels, thus causing the decrease of the Ca^{2+} influx, and consequently with a marked decline of intracellular Ca^{2+}, which is related to cardiac hypertrophy (Godfraind, 1981). It is possible that the regression of cardiac hypertrophy is related to the inhibition of intracellular protein metabolism and oncogeny (*c-myc*, *c-fos*) expression by the declined intracellular Ca^{2+} (Nayler and Grinwald, 1981; Marban and Koretsune, 1990).

The characteristics of the high-affinity site determined by Scatchard analysis fit well with the K_d reported for the high-affinity sites in the left ventricle. Our results let us conclude that only one type of binding site with high-affinity for dihydropyridine was present in the three groups of rats, and was related to the inactivated state of the Ca^{2+} channel (Bean, 1984).

Others have reported that the total number of dihydropyridine binding sites per heart is significantly increased, although the B_{max} of dihydropyridine binding sites remains constant (Mayoux et al., 1988). In this study, we obtained similar findings for the total number of dihydropyridine binding sites per left ventricle from cardiac hypertrophic rats, but the B_{max} of dihydropyridine binding sites was increased in hypertrophic left ventricle induced by DOCA-salt hypertension. The mechanism for this phenomenon, based on the results observed, may be as follows. Firstly, the increased number of Ca^{2+} channels allows an increase in the Ca^{2+} influx necessary for maintaining the contraction of the hypertrophic myocyte and reflects functional adaptation. However, if an increased intracellular Ca^{2+} causes overload, it will do great damage to the energy metabolites and other functions of the cell, including breakdown and depletion of intracellular ATP, loss of mitochondrial ATP-generating ability, and depression of the sarcolemmal enzymatic activity. Some reports have shown that Ca^{2+} channel antagonists might improve energy metabolites and regulate sarcolemmal enzymatic activity by blocking Ca^{2+} influx (Lange et al., 1984; Buser et al., 1989; Chen et al., 1991). In this study, we also found that tetrandrine could markedly decrease myocardial mitochondrial Ca^{2+} in the hypertrophic myocyte and improved the hypertrophic left ventricle compliance (Xu and Rao, 1995).

Secondly, according to the morphometry study of Anyersa et al. (1980), the surface/volume ratio of hypertrophic myocytes remains constant and the surface of the T-tubular system increases approximately 100% in chronically hypertrophic rat hearts. Because of the similar number of binding sites/mg of protein and the constant surface/volume ratio, it can be assumed that the density of Ca^{2+} channels is maintained in the hypertrophic heart by an increased synthesis of channels, particularly in the T-tubular system. The number of functional channels is also increased by pressure overload, thus allowing the increase in the Ca^{2+} influx necessary for maintaining the contraction of the hypertrophic myocyte. This channel synthesis appears to be correlated to the hypertrophy of myocytes and the increase in total heart weight.

Thirdly, it might be possible for these Ca^{2+} domains to increase Ca^{2+} entry through the Ca^{2+} channels by changing their Ca^{2+} sensitivity (Ebata et al., 1991; Mayoux et al., 1988), and the reduced density of Ca^{2+} channels observed with tetrandrine might block Ca^{2+} channels against any cytosolic increase in Ca^{2+}.

It can be concluded that the new Ca^{2+} channel antagonist, tetrandrine, may cause regression of the total cardiac and left ventricular mass and decrease myocardial mitochondrial Ca^{2+} in DOCA-salt hypertensive rats. These effects are accompanied by a reduced density of dihydropyridine binding sites. Therefore, a reduced cardiac pressure overload and a decreased intracellular Ca^{2+} of myocardial mitochondria are the explanation for these effects, and other mechanisms seem to contribute to the regression of cardiac mass.

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