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Action of the calcium channel blocker lacidipine on cardiac hypertrophy and endothelin-1 gene expression in stroke-prone hypertensive rats

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1 The tissue-protective effects of calcium channel blockers in hypertension are not well dissociated from their effect on systolic blood pressure (SBP). We have previously shown that lacidipine, a dihydropyridine-type calcium antagonist, reduced the cardiac hypertrophy and the cardiac endothelin-1 (ET-1) gene overexpression occurring in salt-loaded stroke-prone spontaneously hypertensive rats (SL-SHRSP), an effect occurring without systolic blood pressure (SBP) change. In the present study, we have examined whether this action was dose-related and if it could be associated with ET receptor changes. The action of lacidipine was also examined in control SHRSP and in Wistar Kyoto rats (WKY).

2 The daily dose of 0.3 mg kg^{-1} lacidipine which did not lower SBP but significantly prevented ventricle hypertrophy and cardiac preproET-1-mRNA expression in SL-SHRSP was inactive in control SHRSP. With the higher dose of lacidipine (1 mg kg^{-1} day^{-1}), we observed a further reduction of cardiac hypertrophy and of ET-1 gene expression in SL-SHRSP and a significant effect on those parameters in control SHRSP but only a small reduction of SBP in both groups.

3 In WKY, salt loading did not induce change in SBP or increase of cardiac ET-1 gene expression and ventricle mass. In these normotensive rats, lacidipine (1 mg kg^{-1} day^{-1}) did not modulate the basal preproET-1-mRNA expression and did not affect SBP or heart weight.

4 The maximum binding capacity (B_{max}) and the dissociation constant (K_D) of [^{125}I]-ET-1 binding and the relative proportion of low- and high-affinity binding sites for ET-3 were not significantly affected by salt loading or lacidipine treatment in SHRSP.

5 These results show that lacidipine exerted a dose-related inhibition of ventricle hypertrophy and preproET-1-mRNA expression in SHRSP and indicate that this effect was unrelated to SBP changes. The dose-dependency of this inhibition suggests that salt-induced cardiac hypertrophy could be related to ET-1 gene overexpression. The results further show that ET receptor changes are not involved in the pathophysiological process studied here.

Keywords: Lacidipine; endothelin; cardiac hypertrophy; stroke-prone rats; salt-loading; hypertension; calcium channel blockers

Introduction

Left ventricular hypertrophy (LVH) is identified as a powerful risk factor for myocardial infarction, congestive heart failure and ventricular arrhythmia (Messerli & Ketelhut, 1991). Although hypertension is the leading cause of LVH, the correlation between the arterial pressure and the magnitude of hypertrophy is poor (Drayer *et al.*, 1987). Antihypertensive treatments provide additional evidence that LVH is not exclusively related to an elevated blood pressure. Pure vasodilators like hydralazine do reduce blood pressure of hypertensive patients but not their cardiac hypertrophy (Leenen *et al.*, 1987). By contrast, low doses of angiotensin-converting enzyme (ACE) inhibitors which do not lower elevated systolic blood pressure (SBP), do reduce the increase in cardiac mass (Linz *et al.*, 1991).

Non-haemodynamic determinants, such as sex, race, age, obesity or salt intake have been reported to participate in the cardiac adaptation leading to LVH (for references, see Messerli & Ketelhut, 1991). *In vitro* models are currently used to study the cellular processes by which these factors could regulate the development of cardiac hypertrophy. However, the phenotypic modulation occurring in primary cell culture restricts the extrapolation of the findings to the pathophysiological reality (Morgan & Baker, 1991). Therefore, animal models which allow the *in vivo* analysis of the blood pressure-unrelated components of cardiac hypertrophy are of major interest. We have recently reported that in stroke-prone, spontaneously hypertensive rats (SHRSP), high dietary salt intake induces an overexpression of preproET-1-mRNA and a significant increase in the ventricle:body weight ratio without increasing SBP (Feron *et al.*, 1995a, b). Lacidipine, a powerful dihydropyridine-type calcium antagonist (Salomone & Godfraind, 1993), shown to have tissue-protective effects in hypertension (Gaviraghi & Godfraind, 1993), reduces this salt effect. This long-lasting calcium channel blocker is not only a vasodilator but is also able to reduce the development of cerebral microaneurysms and of cardiac hypertrophy in salt-loaded Dahl-S rats (Cristofori *et al.*, 1991; Gaviraghi *et al.*, 1991). In this study we examined the action of two doses of lacidipine on SBP, cardiac mass and ET-1 gene expression in salt-loaded SHRSP (SL-SHRSP), in control SHRSP (NS-SHRSP) and in Wistar Kyoto rats (WKY).

The results show that lacidipine exerted a dose-related inhibition of ventricle hypertrophy and preproET-1-mRNA expression in SHRSP and indicate that this effect was unrelated to SBP changes. The dose-dependency of this inhibition suggests that salt-induced cardiac hypertrophy could be related to ET-1 gene overexpression. The results also show that ET receptor changes are not involved in the pathophysiological process studied here. Furthermore, the effects described here were not observed in WKY.

Methods

Experimental animals

Successive groups of male SHRSP (Iffa Credo, L'arbresle, France) were used. At the age of 8 weeks, rats were divided at

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random into three subgroups, one control receiving ordinary food and two receiving the same food containing lacidipine for a daily mean intake of 0.3 and 1 mg kg^{-1} respectively. Each of these three subgroups was subdivided in two series, one maintained on 1% NaCl drinking water, called salt-loaded (SL) rats and the other given salt-free water (NS for non-salt loaded). Similarly, at the age of 8 weeks, male Wistar Kyoto rats (WKY) were divided in SL- and NS-series, and within these two series, some received food containing lacidipine (1 mg kg^{-1} day^{-1}) and others ordinary food. Control and lacidipine-treated rats were kept in the same environment and received water and food *ad libitum*. The average daily intake of the diet was measured every day and the SBP was measured every week by the tail-cuff method in conscious animals prewarmed to 35°C in thermostatic cages (Physiograph Narco, Houston, TX, U.S.A.). The total number of rats was 106 SHRSP and 20 WKY.

Rats were killed by decapitation at 14 weeks of age. Hearts were immediately removed and immersed in physiological solution (in mM: NaCl 122, KCl 5.9, $NaHCO_3$ 15, $MgCl_2$ 1.25, $CaCl_2$ 1.25 and glucose 11) maintained at 37°C and aerated with a gas mixture of 95% O_2-5% CO_2. Hearts were dissected free of atria, dried on filter paper and weighted to determine ventricle:body weight ratio. Ventricles were then immediately frozen in liquid nitrogen and stored at −80°C until RNA extraction or tissue homogenization were performed.

Northern blot analysis

Samples of 20 μg of total RNA, isolated from rat ventricles by the guanidinium thyocyanate procedure (Chirgwin *et al.*, 1979), were size-separated on formaldehyde/1% agarose gels and transferred onto HYbond N membranes (Amersham, Little Chalfont, U.K.) by capillary action. After prehybridization for 4 h, blots were hybridized overnight at 65°C to a ^{32}P-labelled random-primed preproendothelin-1 cDNA probe, washed at high stringency and then autoradiographed (Kodak, XAR-film) at −80°C for 24–48 h, as described by Sambrook *et al.* (1989). To ensure that similar amounts of total RNA were compared, the ET-1 probe was stripped by boiling in 0.1 × SSC (1 × SSC = 0.15 M sodium chloride, 0.015 M, sodium citrate, pH 7) solution containing 1% (w/v) sodium dodecyl sulphate (SDS) and the blots were rehybridized to a ^{32}P-labelled rat glyceraldehyde phosphate dehydrogenase (GAPDH) cDNA probe. Prehybridization and hybridization reactions were performed in 50% (v/v) formamide, 5 × SSC, 10 × Denhardt's reagent, 0.5% (w/v) SDS and 100 μg ml^{-1} salmon sperm DNA. Blots were washed three times in 2 × SSC, 0.1% (w/v) SDS at room temperature for 30 min and then in 0.2 × SSC, 0.1% (w/v) SDS at 65°C for 30 min. Densitometric analyses of hybridization signals were performed by scanning autoradiograms with Macintosh-based image-analysis software (Image 1.37, NIH, Bethesda, U.S.A.); optical density (OD) units were normalized with respect to the OD values obtained for the GAPDH internal controls.

Tissue homogenization and radioligand binding experiments

Rat ventricles were finely minced with scissors and homogenized in 20 volumes of a solution containing 250 mM sucrose, 3 mM imidazole, 0.1 mM phenylmethylsulphonylfluoride, 10 mM $MgCl_2$ (pH 7.4 at 2°C), by three 10-s bursts in an Ultra-Turrax (Janke and Kunkel KG., Germany) set at 13,500 r.p.m.. The homogenate was filtered through a 200 μM sieve and centrifuged at 110,000 *g* for 35 min. The resulting pellet was resuspended in homogenization buffer and stored frozen until binding experiments were performed. Protein content was assayed according to Lowry *et al.* (1951) and the DNA content according to Labarca & Paigen (1980).

Membrane aliquots from rat ventricles (30 μg of protein) were incubated at 37°C for 120 min with different concentrations of [^{125}I]-endothelin-1 ([^{125}I]-ET-1) in 250 μl of a buffered solution containing 250 mM sucrose, 3 mM imidazole, 0.1 mM phenylmethylsulphonylfluoride, 0.1 TIU (trypsin inhibitor unit) ml^{-1} aprotinin, 10 mM $MgCl_2$, 1 mg ml^{-1} bovine serum albumin; pH 7.4 at 37°C. In agreement with Ishikawa *et al.* (1991), this time of incubation allowed the development of steady state binding. Non specific binding was measured for each concentration of [^{125}I]-ET-1 by adding cold ET-1 at the final concentration of 0.3 μM. The competition experiments with unlabelled ET-1 and ET-3 were performed with a fixed concentration of 70 pM [^{125}I]-ET-1.

After incubation, suspensions were rapidly filtered on Whatman GF/F filters. Each tube was rinsed twice with 2.5 ml of an ice-cold solution containing 10 mM tris (hydroxymethyl)aminomethane, 50 g l^{-1} polyethylene glycol 6,000, pH 7.4 at 2°C. Filters were washed twice with 10 ml of the same solution. The radioactivity retained on the filters was counted in a γ-counter with an efficiency of about 64%. All determinations were performed at least in triplicate. Binding data were analysed through LIGAND software (Munson & Rodbard, 1980).

Drugs

Non-labelled endothelin-1 and endothelin-3 were obtained from Novabiochem (Läufelfingen, Switzerland); they were dissolved in water as stock solutions at 0.1 mM; [^{125}I]-endothelin-1 (specific activity 2000 Ci $mmol^{-1}$) was obtained from Amersham. Lacidipine was a gift from Glaxo (Verona, Italy) and probe for preproendothelin-1 was provided by Prof. T. Masaki (Kyoto University, Japan).

Statistical analysis

Data are expressed as mean ± standard error (s.e.mean); tests of significance have been made by one-way analysis of variance (ANOVA), *P* values less than 0.05 were considered significant.

Results

Biometric parameters of SHRSP

The measured food intake and the mean body weight were not significantly different between the different series of SHRSP at the end of the 6 weeks of treatment (data not shown). Lacidipine intake was not significantly different between the SL- and NS-SHRSP groups, amounting to 0.28 ± 0.01 and 0.31 ± 0.01 mg kg^{-1} day^{-1} for the lower dose, and to 0.95 ± 0.01 and 1.02 ± 0.01 mg kg^{-1} day^{-1} for the higher dose, respectively. Highly reproducible results in the amount of food and/or lacidipine intake were also found between the different groups of WKY; SL- and NS-WKY received 1.02 ± 0.01 and 1.04 ± 0.01 mg kg^{-1} day^{-1}, respectively. The mean body weight of WKY was 20–30% higher than that of SHRSP. Water intake was almost doubled in salt-loaded SHRSP and WKY, independently of the age and the level of lacidipine intake (not shown).

The age-related increase of SBP in SL-SHRSP was not significantly different from that of NS-SHRSP so that SBP values at the 14th week of age were not significantly different between these two groups. However, salt loading significantly increased cardiac hypertrophy ($P < 0.01$) as shown by the measurements of ventricle:body weight ratio (Figure 1). Lacidipine treatment modulated the evolution of these biometric parameters differently according to the amount of drug intake. Indeed, lacidipine at the daily dose of 1 mg kg^{-1} slightly attenuated the increase in SBP and reduced the ventricle:body weight ratio in both SL- and NS-SHRSP ($P < 0.01$). At the lower dose (0.3 mg kg^{-1} day^{-1}), lacidipine did not show hypotensive effects, reduced cardiac hypertrophy in SL-SHRSP ($P < 0.01$), but was without significant action on heart weight in NS-

SHRSP. In WKY rats, no significant changes in SBP or in ventricle:body weight ratio were induced by salt loading or lacidipine treatment (Table 1).

Northern blot analysis

Northern blot analysis of total RNA extracted from rat ventricles using a specific probe for preproET-1 revealed a single band of 2.3 kb, in agreement with the reported size of preproET-1 transcripts (Sakurai *et al.*, 1991). Densitometric scanning of the autoradiograms allowed us to estimate the relative expression of preproET-1, each band being normalized against the corresponding signal of GAPDH.

In these conditions, the expression of the preproET-1 gene transcript was about 3 fold greater ($P<0.01$) in SL- than in NS-SHRSP (Figure 1). The 0.3 mg kg^{-1} day^{-1} lacidipine regimen significantly prevented the ET-1 gene overexpression in SL-SHRSP ($P<0.01$) but was without effect on preproET-1-mRNA expression in NS-SHRSP (Figure 1).

The higher dose of lacidipine (1 mg kg^{-1} day^{-1}), which, unlike the low dose, slightly reduced the level of SBP ($P<0.01$), not only decreased the ET-1 gene expression in SL-SHRSP but also in NS-SHRSP. The level of preproET-1-mRNA expression attained was significantly lower than that of untreated NS-SHRSP ($P<0.01$) (Figure 1). The ratio of optical densities ET-1/GAPDH was 0.29 ± 0.03 and was similar to that found in WKY (see Table 1).

In WKY rats, lacidipine-treatment (1 mg kg^{-1} day^{-1}) was without effect on the basal preproET-1-mRNA expression and salt loading induced only a non-significant tendency to an increased ET-1 gene expression (Table 1). It is noteworthy that the cardiac mass of WKY was significantly lower than the cardiac mass of SHRSP treated with lacidipine 1 mg kg^{-1} day^{-1} ($P<0.01$) while preproET-1-mRNA levels were similar in both groups.

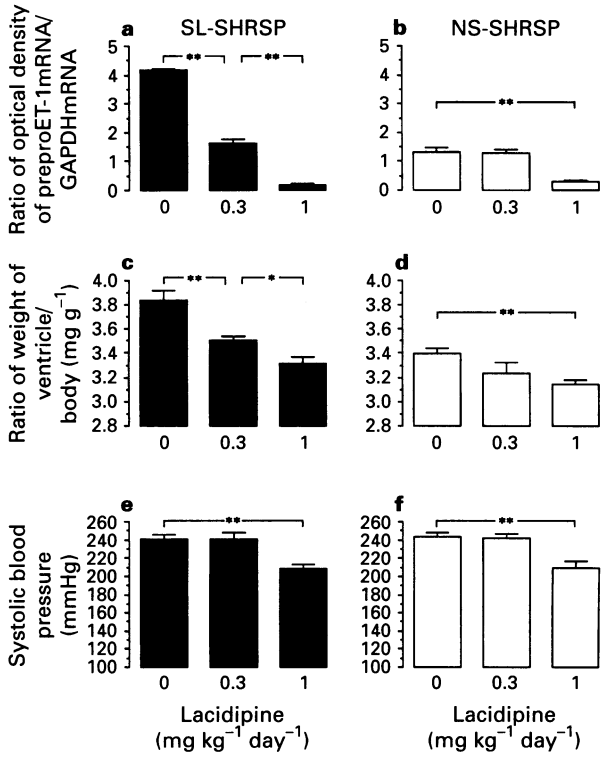

Figure 1 Preproendothelin-1 (ET-1) mRNA content (a,b), cardiac hypertrophy (c, d) and systolic blood pressure (e, f) in salt-loaded (SL) and control (NS) SHRSP, treated or not with lacidipine, as indicated below each column. PreproET-1 mRNA content is expressed as the ratio of the optical density of the preproET-1 mRNA to the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA ($n=3$); cardiac hypertrophy is expressed as the ratio of the ventricle weight to the body weight. For SBP and cardiac mass, $n=$ at least 14. $^*P<0.05$, $^{**}P<0.01$; one way ANOVA.

Characterization of endothelin binding sites

Radioligand binding experiments were carried out in membrane preparations from ventricles of NS-SHRSP and SL-SHRSP with [^{125}I]-ET-1. The specific binding of [^{125}I]-ET-1 (not shown) was saturable with increasing concentrations of the ligand. In both NS- and SL-SHRSP, the Scatchard plots were linear and the Hill plots had a slope close to 1, suggesting the interaction of the radioligand with only one class of binding sites. Table 2 shows the binding parameters of [^{125}I]-ET-1, measured in different membrane preparations from SHRSP. Neither the dissociation constant (K_D) nor the maximum binding capacity (B_{max}) of [^{125}I]-ET-1 binding were significantly affected by salt loading or lacidipine-treatment (1 mg kg^{-1} day^{-1}). Taking into account that the cellular:matrix protein ratio could be different according to the magnitude of cardiac hypertrophy, B_{max} values have been estimated not only in fmol per mg of protein, but also in fmol per g of tissue and in fmol per mg of tissular DNA, this latter being an index of the cell number. As shown in Table 2, B_{max} values were not different between NS- and SL-SHRSP, whether treated or not with lacidipine, even after normalizing for DNA content.

The results of competitive binding inhibition studies in ventricular membranes from NS- and SL-SHRSP are shown in Figure 2. The [^{125}I]-ET-1 binding in both SHRSP populations was completely displaced by either unlabelled ET-1 or ET-3 in a concentration-dependent manner, ET-1 being more potent than ET-3. Moreover, displacement experiments performed with ET-3 showed Hill coefficients lower than 1. When ET-3 competition data were analysed by non-linear regression (Munson & Rodbard, 1980), they were better fitted by a two-site model ($P<0.01$) in both NS- and SL-SHRSP. About 70–80% of ET-3 binding occurred at a high-affinity site, with a K_i in the subnanomolar range; the remaining 20–30% low-affinity binding showed a K_i in the submicromolar range (Table 3). The binding parameters of ET-3 were, like those of ET-1, not significantly different in NS- and SL-SHRSP.

Table 1 Biometric parameters and cardiac preproendothelin-1 (ET-1) mRNA expression of control and salt-loaded Wistar-Kyoto rats, with or without lacidipine 1 mg kg^{-1} day^{-1} treatment

Rats	*Lacidipine-treatment* (mg kg^{-1} day^{-1})	*Ventricle to body weight* (mg g^{-1})	*Systolic blood pressure* (mmHg)	*Ratio of optical density of preproET-1 mRNA GAPDH mRNA*
Control ($n=4$)	0	2.91 ± 0.05	145.3 ± 7.1	0.32 ± 0.12
Control ($n=6$)	1	2.74 ± 0.07	148.2 ± 9.9	0.29 ± 0.11
Salt-loaded ($n=4$)	0	2.93 ± 0.09	152.8 ± 9.2	0.37 ± 0.14
Salt-loaded ($n=6$)	1	3.05 ± 0.06	139.8 ± 3.3	0.34 ± 0.05

Table 2 Characteristics of [^{125}]-endothelin-1 binding to ventricular membranes of control and salt-loaded SHRSP, with or without lacidipine 1 mg kg^{-1} day^{-1} treatment

Rats	*Lacidipine-treatment* (mg kg^{-1} day^{-1})	*Hill coefficient*	K_D (pM)	B_{max} (fmol mg^{-1} protein)	B_{max} (pmol g^{-1} tissue)	B_{max} (fmol μg^{-1}) DNA)
Control ($n=4$)	0	1.03 ± 0.05	48.8 ± 7.9	168.2 ± 21.5	7.72 ± 1.32	12.27 ± 2.47
Control ($n=3$)	1	0.97 ± 0.03	32.3 ± 5.6	174.4 ± 13.7	10.04 ± 1.21	11.14 ± 0.61
Salt-loaded ($n=6$)	0	1.01 ± 0.02	41.8 ± 2.6	159.0 ± 11.2	8.59 ± 0.69	11.55 ± 1.02
Salt-loaded ($n=4$)	1	1.00 ± 0.01	37.0 ± 5.6	196.8 ± 19.8	10.06 ± 0.93	13.03 ± 1.26

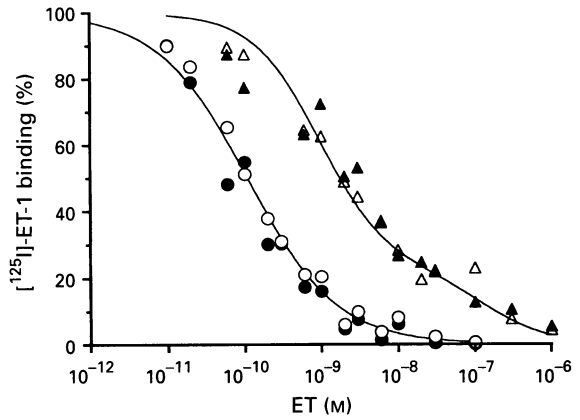

Figure 2 Displacement of [^{125}I]-endothelin-1 ([^{125}I]-ET-1) specific binding by unlabelled endothelin-1 (ET-1, ○, ●) and endothelin-3 (ET-3, △, ▲) from ventricular membranes of NS-SHRSP (open symbols) and SL-SHRSP (solid symbols). Membranes were incubated with 70 pM [^{125}I]-ET-1 and increasing concentrations of unlabelled ET-1 (10 pM – 100 nM) or ET-3 (60 pM – 1 μM). Each point is the mean of 3 determinations, s.e.mean (omitted for clarity) did not exceed 5%. The corresponding binding parameters for ET-1 were: in NS-SHRSP, IC_{50}, 108 pM; K_i, 47.5 pM; in SL-SHRSP, IC_{50}, 95.1 pM; K_i, 39.8 pM. The binding parameters for ET-3 are given in Table 3. The solid lines represent the theoretical displacement curves fitted by a one-site model for ET-1 and a two-site model for ET-3 (Noel & Godfraind, 1984).

Discussion

In this study, we have shown that lacidipine reduced cardiac preproendothelin-1 mRNA expression and ventricular hypertrophy in SHRSP but not in age-matched WKY and that this action was dose-dependent. It is unlikely that it could be related to the well known effect of lacidipine on SBP since 0.3 mg kg^{-1} day^{-1} prevented salt-dependent hypertrophy but was without effect on SBP. Furthermore, lacidipine 1 mg kg^{-1} day^{-1} reduced the preproET-1-mRNA content down to the level found in WKY whereas the SBP was still much higher in SHRSP than in WKY.

Reduced increase in cardiac mass accompanied by reduced preproET-1-mRNA expression (Figure 1) indicates that autocrine/paracrine ET-1 secretion pathway in SHRSP could be a factor in the proliferative process blunted by lacidipine. But other factors could coexist since the cardiac mass of SHRSP treated by lacidipine 1 mg kg^{-1} day^{-1} was higher than the cardiac mass of WKY with a similar content of preproET-1-mRNA. It could be that the still high SBP was responsible for this hypertrophy. This could account for the observation that ET-1 antagonists reduce this hypertrophy only partly (Stasch *et al.*, 1995). Schiffrin (1995) recently reported that ET-1 is involved in vascular hypertrophy occurring in deoxycorticosterone acetate-salt hypertensive rats and that bosentan, a non-selective ET receptor antagonist, reduced this hypertrophy. Also, Ito *et al.* (1994) reported that BQ123, an ET_A receptor antagonist, blocked cardiac hypertrophy provoked in rats by haemodynamic overload. Blunting of ET-1 gene expression could therefore account for the cardiac and vascular anti-hypertrophic action of lacidipine (this study and Feron *et al.*, 1995b). Possible mechanisms for the ET-1 gene regulation by lacidipine could be considered on the basis of the existence of two putative binding sites identified in the promoter sequence of these gene (Paul *et al.*, 1995). The presence of a calcium-responsive element indicates that the preproET-1-mRNA production is highly sensitive to calcium concentration and therefore, could be directly modulated by the lacidipine-evoked calcium entry blockade. Also, the influx of calcium through L-type calcium channels is known to trigger *c-fos* activation (Murphy *et al.*, 1991; Mizra *et al.*, 1994) and lacidipine could therefore indirectly modulate the interaction of the product of this immediate early gene with the AP-1 binding site present in the promoter sequence of ET-1 gene. Other mechanisms unrelated to the L-type calcium channel blockade could also account for the inhibition of gene expression by lacidipine. For instance, the high partition of lacidipine into biomembranes (Herbette *et al.*, 1993) could affect the properties of some membrane proteins involved in the modulation of gene expression (Roth *et al.*, 1992). Moreover, the free radical scavenging properties of lacidipine (van Amsterdam *et al.*, 1992) could reduce free radicals which are known to induce ET-1 production in myocardium (De Keulenaar *et al.*, 1995).

The absence of modulation of preproET-1-mRNA expression by lacidipine (1 mg kg^{-1} day^{-1}) in the heart of normotensive WKY rats suggests that this drug did not interact with the basal level of ET-1 gene expression. In these rats, salt loading did not induce changes in SBP or in the ventricle mass and showed only a non-significant tendency to an increase of preproET-1-mRNA expression. These data indicate that lacidipine regulated ET-1 gene expression through the blockade of a cellular process which occurred in the SHRSP malignant hypertensive model and was augmented by salt loading.

Different factors have been implicated in the pathogenic process of tissue injury and remodelling in SHRSP; growing evidences suggest that the renin-angiotensin system (RAS) could be involved. Paradoxical elevated plasma renin activity has been reported in SHRSP exposed for several weeks to a high-salt intake (Shibota *et al.*, 1979; Volpe *et al.*, 1990; Stier *et al.*, 1991). Angiotensin II (AII) is involved in the development of tissue lesions in SHRSP as indicated by the protective action of enalapril and captopril (Stier *et al.*, 1989; 1991) and of AII type I receptor antagonist (Kim *et al.*, 1995). Nara *et al.* 1991 have reported that a restriction fragment length polymorphism of the ACE gene cosegregates with the blood pressure level in SHRSP. Furthermore, recent studies established a link between the RAS and ET system. Indeed, AII was shown to stimulate the release of immunoreactive ET-1 in rat vascular smooth muscle (Sung *et al.*, 1994) and to induce up-regulation of both preproET-1 and ET_B receptor mRNA in neonatal rat cardiomyocytes (Ito *et al.*, 1993; Kanno *et al.*, 1993). Moreover, Ito *et al.* (1993) demonstrated that AII-induced hypertrophy of rat cultured cardiomyocytes is partly blocked by an ET receptor antagonist. Since ET-1 *per se* stimulates hypertrophy in rat cardiomyocytes in culture (Suzuki *et al.*, 1990; Ito

Table 3 Characteristics of endothelin-3 binding to ventricular membranes of control and salt-loaded SHRSP

	Hill coefficient	K_{iH}[a] (pM)	K_{iL}[b] (nM)	B_{maxH}[c] (%)	B_{maxL}[d] (%)
Control ($n=3$)	0.56±0.03	552±127	134±55	81.5±3.6	18.5±3.6
Salt-loaded ($n=3$)	0.46±0.03	469±174	141±55	71.0±8.1	29.0±8.1

In all preparations tested, the displacement of [^{125}I]-ET-1 binding by ET-3 was better fitted by a two-site model than by a one-site model ($P<0.001$). [a] Dissociation constant for the high affinity site. [b] Dissociation constant for the low affinity site. [c] Maximum binding capacity for the high affinity site. [d] Maximum binding capacity for the low affinity site.

et al., 1991) and since an ET_A receptor antagonist is able to block cardiac hypertrophy *in vivo* (Ito *et al.*, 1994), ET-1 could be the final mediator of cardiac hypertrophy. Therefore, we may speculate from our findings that by blunting the angiotensin-operated cellular pathway leading to preproET-1-mRNA expression, which is directly related to the ET-1 peptide production (Larivière *et al.*, 1993; Schiffrin, 1995), lacidipine could prevent cardiac hypertrophy in SHRSP. Alternatively, lacidipine could attenuate the paradoxical increase in plasma renin activity, since calcium antagonists, inhibit renin production in some experimental models (Godfraind *et al.*, 1986; Shudo *et al.*, 1994). However additional studies are required to verify the present hypothesis involving the RAS in the interaction of lacidipine with the cardiac ET system.

We have also observed that the density of ET specific binding sites in cardiac tissues was not modulated in SHRSP by salt diet or lacidipine treatment. Both ET_A and ET_B receptors are known to be widely distributed throughout the myocardium (Hori *et al.*, 1992; Molenaar *et al.*, 1993; Peter & Davenport, 1995) but little information is available about the regulation of cardiac ET receptors. Down-regulation of ET receptors has been reported after prolonged ET-1 exposure in vascular smooth muscle cells (Hirata *et al.*, 1988) and osteosarcoma cell line (Sakurai *et al.*, 1992). By contrast, in our experiments, despite the induction of ET-1 gene expression by salt loading, no modification of the maximal binding capacity of ET-1 was observed (Table 2). Furthermore, the relative proportion of low- and high-affinity binding sites for ET-3 was not altered. Different hypotheses may be formulated to explaint this discrepancy between *in vitro* and *in vivo* observations. First, the level of ET-1, albeit increased in salt-loaded SHRSP, could remain insufficient to induce a decrease of ventricle ET receptor density. Secondly, we cannot exclude the possibility that changes in ET receptor characteristics may have occurred in specific cell types of the myocardium and were not detected by radioligand binding experiments performed on total ventricle tissues. Moreover, differences in the regulation of ET receptors among tissues and models of hypertension have been reported (Haizer *et al.*, 1994) and could account for the absence of modulation in SHRSP ventricle tissues. Fu *et al.* (1993) observed a decreased density of ET receptors in mesenteric arteries but not in myocardium of rats with chronic ischaemic heart failure which is known to be accompanied by increased ET-1 immunoreactivity. Furthermore, it has been reported that some stimuli, such as AII, induce ET-1 overexpression and up-regulation of ET receptors (Ito *et al.*, 1993; Kanno *et al.*, 1993).

In summary, this study shows that the chronic administration of the calcium antagonist, lacidipine, dose-dependently reduced salt-dependent ET-1 gene expression and concomitant cardiac hypertrophy. Further studies will be required to elucidate the molecular mechanism of those actions and to provide insights into the potential link between the renin-angiotensin and ET systems in order to characterize better the processes activated by high salt intake.

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