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INHIBITION BY LACIDIPINE OF SALT-DEPENDENT CARDIAC HYPERTROPHY AND ENDOTHELIN GENE EXPRESSION IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

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Stroke-prone spontaneously hypertensive rats receiving a high salt diet were orally treated by the calcium antagonist lacidipine, at a dose which did not reduce systolic blood pressure. We observed that lacidipine inhibited the salt-induced cardiac hypertrophy and the concomitant increase of mRNA transcripts for preproendothelin-1 in ventricles. These data show that elevated blood pressure cannot necessarily account for cardiac hypertrophy and indicate that the therapeutic action of lacidipine is not only related to its haemodynamic properties, but also to the inhibition of the gene expression of growth factors such as endothelin.

We have recently shown that, in stroke-prone spontaneously hypertensive rats (SPSHR), sodium has a trophic effect on cardiac mass, which is superimposed on that of the pressure load imposed on the left ventricle in essential hypertension, and that it increases the cardiac expression of the preproendothelin-1 mRNA, indicating that the expression of growth factors could be involved in this process (1). L-type calcium channel blockers are known to reduce cardiac hypertrophy accompanying essential hypertension but this cardio-protective effect has not been dissociated from their antihypertensive properties (2). Therefore, salt-loaded stroke-prone rats appeared to be an appropriate model in order to investigate the potential inhibition, by calcium antagonists, of the two aforementioned major cardiovascular changes occurring in

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ABBREVIATIONS: ET-1, endothelin-1; SBP, systolic blood pressure; SPSHR, stroke-prone spontaneously hypertensive rat; SL, salt-loaded; NS, non-salt-loaded; GAPDH, glyceraldehyde phosphate dehydrogenase.

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hypertension. For this purpose, we have used lacidipine, a selective calcium antagonist endowed with potent and long lasting antihypertensive actions (3). Several reports have shown that lacidipine has protective effects against stroke at low doses which do not modify elevated systolic blood pressure (SBP) (4, for references).

We have therefore examined the effect of a low dose of lacidipine on cardiac hypertrophy and endothelin-1 (ET-1) gene expression production in salt-loaded stroke-prone spontaneously hypertensive rats (SPSHR-SL). The results show that a drug regimen which did not change SBP, reduced salt-dependent cardiac hypertrophy and the concomitant increased expression of the preproET-1 mRNA.

MATERIALS AND METHODS

Experimental animals. At age of 8 weeks, SPSHR (Iffa Credo, L'Arbresle, France) were divided at random in two groups, one control group receiving ordinary food and the other receiving the same food containing lacidipine for a daily mean intake of 0.3 mg kg^{-1}, a dose known, in this animal model, to be ineffective in controlling SBP (4). Each group was subdivided in two series, one maintained on 1% NaCl drinking water, named salt-loaded (SL) rats and the other was given salt-free water (NS for non-salt-loaded). Control and lacidipine-treated rats were kept in the same environment and received water and food ad libitum. SBP was measured every week by the tail-cuff method in conscious animals prewarmed to 35°C in thermostatic cages (Physiograph Narco, Houston, TX, USA). Considering that SPSHR show a maximal life-span of 12 weeks after the beginning of a salt-rich diet and generally begin to die after 7-8 weeks of this treatment (4), rats were killed in this study by decapitation after 6 weeks of treatment.

Tissue samples. Heart and thoracic aorta were immediately removed, cleaned of connective tissue and immersed in physiological saline solution containing (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 gassed with 95% O_2 and 5% CO_2. Hearts were dissected free of atria, dried on filter paper and weighed to determine ventricle:body weight ratio. Ventricles were then immediately frozen in liquid nitrogen and stored at -80°C until RNA extraction was performed. Aorta rings were weighed and their length was measured with a binocular microscope.

Northern blot analysis. 20 μg of total RNA isolated from rat ventricles by the guanidinium thyocyanate procedure (5), was electrophoresed through a formaldehyde/agarose gel and transferred onto HYbond N membranes (Amersham). Blots were hybridized to ^{32}P-labelled random-primed preproendothelin-1 cDNA probe, washed at high stringency and then autoradiographed at -80°C for 24 hours, as described by Sambrook et al. (6). To ensure that similar amounts of total RNA were compared, blots were rehybridized with a ^{32}P-labelled rat glyceraldehyde phosphate dehydrogenase (GAPDH) cDNA probe. Densitometric analyses of hybridization signals were performed by scanning autoradiograms;

arbitrary optical density (OD) units were normalized with respects to the OD values obtained for the GAPDH internal controls.

Statistical analysis. Data are expressed as means ± s.e.mean. Tests of significance have been made by analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The age-related increase of SBP was not altered by salt loading and/or lacidipine treatment as shown by the SBP values in the different groups of SPSHR at the 14th week of age (Table 1). Confirming our previous data, we observed that salt loading significantly induced cardiac and aortic hypertrophy ($P < 0.01$) in SPSHR. Furthermore, we noticed that lacidipine, at the daily oral dose of 0.3 mg kg^{-1}, reduced this cardiovascular hypertrophy in SPSHR-SL ($P < 0.05$), but was without significant action on ventricle and aorta weights in SPSHR-NS (Table 1). This confirms that the protective properties of lacidipine are not necessarily related to a reduction of SBP. Moreover, since no significant effect on cardiac mass was perceived in non-salt-loaded rats, this indicates that this low dose of lacidipine specifically blunted salt-dependent mechanisms.

Table 1. Biometric parameters of non-salt-loaded (NS) and salt-loaded (SL) stroke-prone spontaneously hypertensive rats (SHRSP)

	Blood pressure (mm Hg)	Ventricle to body weight ratio (mg/g)	Aorta weight/length (mg/mm)
SHRSP-NS			
Lacidipine			
0 (n = 7)	235.9 ± 9.3	3.28 ± 0.05	0.789 ± 0.021
0.3 mg kg^{-1} day^{-1} (n = 7)	241.9 ± 6.1	3.15 ± 0.05	0.770 ± 0.028
SHRSP-SL			
Lacidipine			
0 (n = 7)	241.9 ± 16.2	3.75 ± 0.12**	0.918 ± 0.026**
0.3 mg kg^{-1} day^{-1} (n = 7)	264.1 ± 10.2	3.40 ± 0.06††	0.840 ± 0.020†

** $P < 0.01$, vs. SHR-NS lacidipine 0; † $P < 0.05$, †† $P < 0.01$, vs. SHR-SL lacidipine 0.

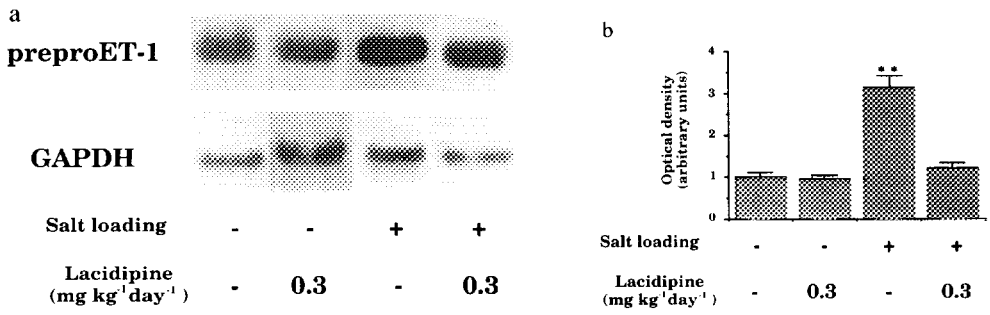

Figure 1.

a. Representative Northern blot analysis of total RNA (20 µg/lane), extracted from ventricles of SPSHR, hybridized with ^{32}P-labeled cDNA probe for rat preproendothelin-1 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The effects of salt loading and/or lacidipine daily treatment are represented.

b. Bar graph shows relative optical density values (± s.e.m.) of the 2.3 kb-preproET-1 mRNA band obtained from different animals (n=3) and normalized against the corresponding signal of GAPDH. The intensity of the preproET-1 band in lanes corresponding to total RNA from lacidipine-untreated non-salt-loaded SPSHR was fixed equal to the unity.

** $P < 0.01$ versus non-salt-loaded SPSHR.

Northern blot analysis of total RNA extracted from rat ventricles using a specific probe for preproendothelin-1 revealed a single band of 2.3 kb in agreement with the size of preproET-1 transcripts previously described (7). Densitometric scanning of the autoradiograms showed that the expression of the preproET-1 gene transcript was 3.2-fold greater ($P < 0.01$) in 14-weeks-old SPSHR-SL than in age-matched SPSHR-NS (Figure 1), in good agreement with our previous study (1). But the most interesting finding of the present study was that lacidipine treatment prevented this increase. At the daily dose of 0.3 mg kg^{-1}, lacidipine reduced the salt-dependent preproET-1 mRNA expression close to the level of untreated SPSHR-NS (Figure 1).

This suggests that the reduction of cardiac hypertrophy by lacidipine could be related to the reduction of ET-1 expression. As lacidipine is a highly selective calcium antagonist, it is tempting to propose that Ca^{2+} entry blockade through the plasma membrane could be the mechanism underlying this inhibition. Calcium influx is known to be involved in the regulation of immediate early gene transcription induced by ET-1 and Ang II (8). It may be that lacidipine, by regulating calcium fluxes across the plasma membrane, could inhibit the salt-dependent induction of ET-1

gene. If this view is correct, other calcium antagonists should exert similar effects but this has not yet been reported.

Alternatively, so-called secondary properties of L-type calcium channel blockers could account for the inhibition of ET-1 expression in ventricles of lacidipine-treated rats. For instance, perturbation resulting from the interaction of a highly lipophilic drug such as lacidipine with the plasma membrane may physically affect the activity of membrane proteins. Roth et al. (9) have reported that the protein kinase C signal transduction pathway, known to cause protooncogenes expression, could be perturbated by a high concentration of a dihydropyridine into lipid bilayers. It will be necessary to examine the effect of lacidipine on endothelin-1 gene expression in isolated cells in order to see if the action here reported is a direct one or is mediated through a neuroendocrine pathway sensitive to both salt loading and lacidipine.

In summary, these data confirm our previous finding that salt-dependent cardiac hypertrophy could be related to the increase of mRNA transcripts for preproET-1 (1) and they show that lacidipine prevented both phenomena. This points out the fact that elevated SBP cannot necessarily account for cardiac hypertrophy and overproduction of ET-1 in the heart of salt-loaded rats. This indicates that the therapeutic action of lacidipine may be related to both haemodynamic properties and inhibition of the gene expression of growth factors such as ET-1.

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