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INFLUENCE OF SALT LOADING ON THE CARDIAC AND RENAL PREPROENDOTHELIN-1 mRNA EXPRESSION IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

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Endothelin-1 is a potent vasoactive peptide which may play a role in the regulation of vascular resistance through its autocrine/paracrine effects. We have investigated the influence of salt loading on the renal and cardiac production of endothelin-1 in stroke prone spontaneously hypertensive rats, a classical model of hypertension. The results show that the dietary salt intake did not change systolic blood pressure nor the renal expression of the preproendothelin-1 mRNA but increased cardiac expression of the endothelin-1 gene transcript and a concomitant ventricular hypertrophy.

Endothelin-1 (ET-1) is a 21-amino-acid residue vasoactive peptide originally characterized from the supernatant of cultured porcine aortic endothelial cells (1). It has been proposed that ET-1 may play a role in the pathophysiology of hypertension but plasma ET-1 concentrations in experimental and human hypertension are similar or slightly higher than in normotensive controls (2, for references). Kurihara et al. (3) have recently reported that the ET-$1^{+/-}$ heterozygous mices, which produce lower plasma levels of endothelin-1 than the wild-type mice, develop elevated blood pressure. It could therefore be that ET-1 plays a more important role in the regulation of vascular resistance as an autocrine/paracrine effector than as a circulating factor.

Among effects which would not be mediated through circulating ET-1, pathophysiological involvement of ET-1 production has recently been

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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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suggested in the renal and cardiac tissues. Hoffman et al. (4) have claimed that, because of the diuretic and natriuretic effect of endothelin (5, 6), its is conceivable that a renal deficiency of the peptide causes volume-overload hypertension. As for the role of ET-1 in the heart, ET-1 was shown to stimulate hypertrophy and contractility of cultured rat cardiomyocytes (7), associated with the induction of muscle-specific gene transcripts (8).

Moreover, an association between salt sensitivity and a greater incidence of renal failure and cardiac hypertrophy has been described in some groups of hypertensive patients (9,10). In this study, we have therefore investigated the influence of a 6-weeks duration of salt loading on the renal and cardiac production of ET-1 in stroke prone spontaneously hypertensive rats (SPSHR). The results show that the dietary salt intake did not augment systolic blood pressure (SBP) neither the renal expression of the preproendothelin-1 mRNA but increased the cardiac expression of the ET-1 gene transcript and a concomitant ventricular hypertrophy.

MATERIALS AND METHODS

Experimental animals. At age of 8 weeks, SPSHR (Iffa Credo, L'arbresle, France) were divided at random in two groups, one maintained on 1% NaCl drinking water, called salt-loaded (SL) rats and the other was given salt-free water (NS for non-salt-loaded). SL- and NS-rats were kept in the same environment and received water and food ad libitum. The systolic blood pressure (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 (11), rats were killed in this study by decapitation after 6 weeks of salt loading.

Tissue samples. Heart and kidneys were immediately removed, cleaned of connective tissue 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 weighed to determine venticle:body weight ratio. Ventricles and kidneys were then immediately frozen in liquid nitrogen and stored at -80°C until RNA extraction was performed.

Northern blot analysis. 20 µg of total RNA isolated from rat ventricles or kidneys by the guanidinium thyocyanate procedure (12), 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 (13). 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 autoradiographs; 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.m.; tests of significance have been made by analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The age-related increase of SBP in SL-SPSHR was not different from that of NS-SPSHR so that blood pressure values after 6 weeks of NaCl treatment were not significantly different between these two groups (Table 1). At the opposite, the salt loading significantly induced cardiac hypertrophy as shown by the measurements of ventricle:body weight ratio (Table 1).

Northern blot analysis of total RNA extracted from rat ventricles and kidneys 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 (1). Densitometric scanning of the autoradiograms showed that the cardiac expression of preproET-1 mRNA was 3.4 fold more elevated in 14-weeks-old SPSHR-SL than in age-matched SPSHR-NS ($P < 0.01$). No significant difference in the expression of the gene of preproET-1 was detected from the kidney of these two types of rats (see Figure 1). This finding together with the observation that the SBP was not significantly different in each group is in agreement with the hypothesis proposed by Hoffman et al. (4) that the level of renal ET-1 is closely related to the blood pressure level.

But a most interesting conclusion from our study is that the SBP modulation may not be advanced to explain the production of ET-1 in salt-loaded rat ventricles and the significant increase of cardiac hypertrophy in these same rats. In different publications (10, for references), it has

Table 1. Biometric parameters of SPSHR-NS AND -SL

	Body weight (g)	SBP (mm Hg)	Ventricle:body weight ratio (mg/g)
SPSHR-NS (n=8) (14-weeks-old)	253.5 ± 4.6	234.6 ± 8.0	3.17 ± 0.03
SPSHR-SL (n=8) (14-weeks-old)	262.1 ± 5.6	226.1 ± 11.6	3.43 ± 0.04 *

(*) $P < 0.01$ vs SPSHR-NS.

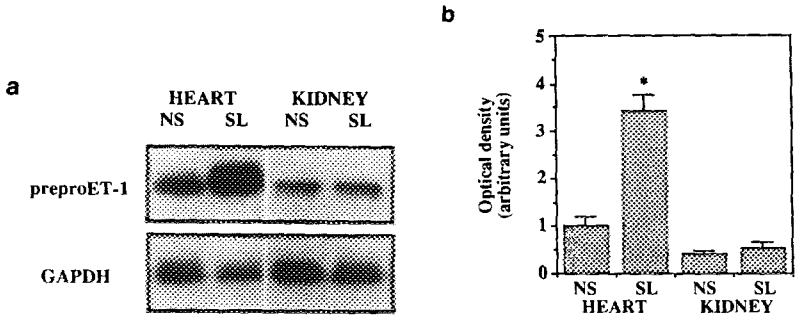

Figure 1. Analysis of preproET-1 mRNA expression in SPSHR.
a. Representative Northern blot analysis of total RNA (20 µg/lane), extracted from ventricles and kidneys of salt-loaded (SL) and non-salt-loaded (NS) SPSHR, hybridized with [^{32}P]-labeled cDNA probe for rat preproendothelin-1 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH).
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. *P < 0.01 versus ventricles of SPSHR-NS.

been suggested that a trophic effect of sodium on cardiac mass may be superimposed on that of pressure load imposed on left ventricle in essential hypertension. From our data, ET-1, which is known to act in a paracrine/autocrine manner in growth regulation through induction of immediate-early genes (14), could therefore be considered as one of the missing links between dietary salt intake and BP-independent cardiac hypertrophy. We cannot exclude that, in this model of genetically hypertensive rats, the cardiac ET-1 level is not in part dependent on the SBP level. Nevertheless, Larivière et al. (15) showed that vascular hypertrophy and increased production of immunoreactive ET-1 were found in the vessels of salt-loaded mineralocorticoid-dependent hypertensive rats and not of spontaneously hypertensive rats, but they have not provided information on renal ET-1 expression.

The mechanism for the salt-induced production of ET-1 in the heart of SPSHR may be related to any of the different stimuli that increase the expression of ET-1 mRNA transcripts. Interestingly, Ang II-induced hypertrophy of cultured rat cardiomyocytes was shown to be partially blocked by an endothelin receptor antagonist (16). Moreover, Sung et al. (17) have recently demonstrated that Ang-II stimulated the release of immunoreactive endothelin from cultured vascular smooth muscle cells. Cardiac-specific regulation of the renin-angiotensin system could therefore mediate local responses such as the induction of ET-1 production. However, dietary salt excess is known to reduce plasma renin

activity in SHRSP (18) and this could be the same for the tissular renin activity although enhancement of brain renin-angiotensin system following long-term salt loading has been reported in spontaneously hypertensive rats (19). Thus, the role of tissular renin-angiotensin system needs still to be better characterized after salt-loading.

In summary, we have demonstrated that the expression of preproET-1 mRNA is differentially modulated by salt loading in the heart and the kidneys of SPSHR, suggesting a tissue-specific regulation of ET-1 production. This strengthens the involvement of ET-1 in autocrine/paracrine pathways and justifies a possible therapeutic approach using specific endothelin receptor antagonist for salt-dependent pathological mechanisms accompanying or responsible for essential hypertension.

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