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Effects of α-lipoic acid on deoxycorticosterone acetate–salt-induced hypertension in rats

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Abstract

We investigated the potential of natural occurring antioxidant α-lipoic acid to prevent hypertension and hypertensive tissue injury induced by deoxycorticosterone acetate (DOCA) and salt in rats. Two weeks after the start of DOCA–salt treatment, the rats were given α-lipoic acid (10 or 100 mg/kg/day, s.c.) or its vehicle for 2 weeks. Uninephrectomized rats without DOCA–salt treatment served as sham-operated controls. In vehicle-treated DOCA–salt rats, systolic blood pressure increased markedly after 3–4 weeks. Daily administration of 100 mg/kg α-lipoic acid for 2 weeks suppressed the increase in systolic blood pressure, whereas 10 mg/kg α-lipoic acid did not affect the progression of DOCA–salt-induced hypertension. When the degree of vascular hypertrophy of the aorta was morphometrically evaluated at 4 weeks, there were significant increases in media cross-sectional area in vehicle-treated DOCA–salt rats compared with sham-operated rats. The development of vascular hypertrophy was markedly suppressed by α-lipoic acid at 100 mg/kg but not at 10 mg/kg. Histopathological examination of the kidney in vehicle-treated DOCA–salt rats revealed fibrinoid-like necrosis in glomeruli and thickening of small arteries. In these animals, creatinine clearance decreased, and fractional excretion of Na^+, urinary excretion of protein and *N*-acetyl-β-glucosaminidase increased. Such renal lesions and dysfunctions were ameliorated in DOCA–salt rats given α-lipoic acid. In addition, a marked increase in endothelin-1 content in both the aorta and kidney was evident in vehicle-treated DOCA–salt rats compared with findings in sham-operated rats. Significant attenuation of this increase occurred in α-lipoic acid-treated DOCA–salt rats. These results suggest that administration of α-lipoic acid to DOCA–salt hypertensive rats lessens the increased blood pressure and protects against renal and vascular injuries, possibly through the suppression of renal and vascular endothelin-1 overproduction.

Keywords: α-Lipoic acid; Deoxycorticosterone–salt; Hypertension; Vascular hypertrophy; Renal injury; Endothelin-1

1. Introduction

α-Lipoic acid is a cofactor in the multienzyme complexes that catalyze the oxidative decarboxylation of α-keto acids such as pyruvate and α-ketoglutarate (Reed, 1974). α-Lipoic acid has also been demonstrated to quench radicals, exhibit chelating, and participate in its reduced form dihydrolipoic acid in the regeneration of ascorbate and vitamin E (Packer et al., 1995). This naturally occurring antioxidant has been proposed to be a potential therapeutic agent in the treatment or prevention of different pathologies including diabetes, polyneuropathy, cataracts, ischemia/reperfusion and neurodegeneration (Packer et al., 1995).

It has furthermore been shown that α-lipoic acid reduces activation of nuclear factor kappa B (NF-κB), a transcriptional factor (Suzuki et al., 1992; Packer, 1998). Bierhaus et al. (1997) noted that advanced glycation end product-induced activation of NF-κB is suppressed by α-lipoic acid in cultured endothelial cells, and this effect is accompanied by reduced transcription and expression of endothelial genes such as tissue factor and endothelin-1. More recently, they identified an NF-κB binding site in the human endothelin-1 gene and confirmed that endothelin-1 transcription is controlled by NF-κB in advanced glycation end product-stimulated cultured endothelial cells (Quehenberger et al., 2000). We also have obtained findings that in cultured aortic endothelial cells, α-lipoic acid partially suppresses basal endothelin-1 release and completely suppresses tumor necrosis factor-α (TNF-α)-induced endothelin-1 release (Ohkita et al., unpublished data). These findings raise the possibility that administration of α-lipoic acid to experimental animals with enhanced endothelin-1 production might ameliorate the endothelin-1-related impairment. To examine this, we uti-

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lized deoxycorticosterone acetate (DOCA)–salt hypertensive rats. This model of hypertension is associated with markedly elevated endothelin-1 content and its mRNA expression in vascular and renal tissues (Larivière et al., 1993a,b; Fujita et al., 1995, 1996a; Deng et al., 1996), and thus provides an opportunity to study the effect of α-lipoic acid on the production of endothelin-1 in vivo. In the present study, we investigated the effects of chronic treatment with α-lipoic acid on the development of hypertension, vascular hypertrophy and renal injury in DOCA–salt hypertensive rats.

2. Materials and methods

2.1. Animals and experimental design

Male Sprague–Dawley rats (SLC, Hamamatsu, Japan), weighing 180–200 g, were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and the right kidney of each rat was excised through a right flank incision. After a 1-week recovery period, rats were separated into a sham-operated group and a DOCA–salt group. The latter was treated twice weekly with DOCA suspended in corn oil, which was administered subcutaneously (15 mg/kg), and 1% NaCl was added to their tap water for drinking. The sham group was not given DOCA or salt. Two weeks after the start of DOCA–salt treatment, animals were assigned to one of five study groups: two sham-operated groups receiving vehicle or α-lipoic acid (100 mg/kg) and three DOCA–salt groups receiving vehicle or α-lipoic acid (10 or 100 mg/kg). α-Lipoic acid or vehicle, in a volume of 1 ml/kg, was administered subcutaneously once daily for 2 weeks. Systolic blood pressure was monitored once a week by the tail-cuff method, before drug administration. Two weeks after the start of drug administration, urine was collected overnight by housing the animals in individual metabolic cages (after the final drug treatment). After urine collection, all rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), exsanguinated, and arterial blood samples were obtained. The left kidney and the descending aorta (4 cm) from just below the aortic arch were also excised.

2.2. Analytical procedures

Creatinine levels in plasma or urine and urinary protein were determined with the Creatinine-test-Wako and Total protein-test-Wako (Wako, Osaka, Japan), respectively. Urinary *N*-acetyl-β-glucosaminidase activity was measured as an index of damage to the proximal tubules, using the synthesized substrate sodio-*m*-cresolsulfonphthalinyl *N*-acetyl-β-D-glucosaminide. Urine and plasma sodium concentrations were determined with a flame photometer (Hitachi, 205D, Hitachi, Japan). Fractional excretion of sodium was calculated from the formula $FE_{Na} = U_{Na}V/(P_{Na} \times \text{creatinine clearance}) \times 100$, where $U_{Na}V$ is urinary excretion of sodium and P_{Na} is plasma sodium concentration.

2.3. Histological studies

The upper part of the descending aorta (2 cm) and left kidney of each rat was preserved in phosphate-buffered 10% formalin, after which the tissues were chopped into small pieces, embedded in paraffin, cut at 4 μm, and stained with hematoxylin and eosin. Four different cross-sections of each vessel placed under a microscope were photographed, and vessel wall, wall thickness, and wall-to-lumen ratio were determined with an image analyzer (ATTO, AE-6905C, Tokyo, Japan). The cross-sectional area (wall area: S) of the vessels was calculated as: $S = \pi M(\mathrm{ED} - M)$, where M is wall (media) thickness and ED is the external diameter. ED was calculated as: $\mathrm{ED} = L_e/\pi$. M was calculated as: $M = (L_e - L_i)/2\pi$. L_e and L_i are the total lengths of the adventitia and the internal elastic membrane, respectively.

2.4. Endothelin-1 measurement

Endothelin-1 was extracted from the lower part of the descending aorta (2 cm) and kidney, according to the method of Fujita et al. (1995). Briefly, aortas and kidneys were rapidly cleaned of fat and adherent connective tissue, weighed and homogenized for 1 min in ice-cold organic solution (chloroform/methanol, 2:1, including 1 mM *N*-ethylmaleimide). The homogenates were left overnight at 4 °C, then 0.4 ml of distilled water was added. The samples were then centrifuged at 3000 rpm for 30 min and the supernatant was stored. Aliquots of the supernatant were diluted 1/10 with a 0.09% trifluoroacetic acid solution and applied to Sep-Pak C_{18} cartridges. The sample was eluted with 3 ml of 63.6% acetonitrile and 0.1% trifluoroacetic acid. Eluates were dried in a centrifugal concentrator and the dried residue was reconstituted in assay buffer for radioimmunoassay. Recoveries of endothelin-1 from tissues in our extraction procedures were approximately 80%. Radioimmunoassay for endothelin-1 was performed as described elsewhere (Matsumura et al., 1990). The limit of detection of endothelin-1 in this assay was 3 pg/tube. Endothelin-1 antiserum (a generous gift from Dr. Marvin R. Brown, Department of Medicine, University of California, San Diego, CA, USA) does not cross-react with big endothelin-1, as described previously (Hexum et al., 1990).

2.5. Drugs

α-Lipoic acid, purchased from Nacalai Tesque (Kyoto, Japan), was dissolved in a solution consisting of 20% ethanol and 80% corn oil. Other chemicals were obtained from Nacalai Tesque and Wako.

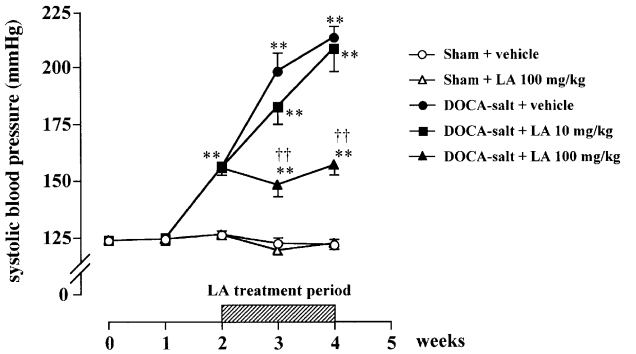

Fig. 1. Time course in systolic blood pressure of sham-operated and deoxycorticosterone acetate (DOCA)–salt rats treated with vehicle or α-lipoic acid (LA). Each point and bar represents the mean ± S.E.M ($n = 6$). $^{**}P < 0.01$, compared with vehicle-treated sham rats. $^{\dagger\dagger}P < 0.01$ compared with vehicle-treated DOCA–salt rats.

2.6. Statistical analysis

Values are expressed as means ± S.E.M. For statistical analyses, we used one-way analysis of variance followed by Bonferroni's multiple comparisons test. For all comparisons, differences were considered significant at a value of $P < 0.05$.

3. Results

3.1. Effects of treatment with α-lipoic acid on blood pressure of DOCA–salt hypertensive rats

As shown in Fig. 1, treatment with DOCA and salt for 2 weeks led to a mild but significant hypertension, and thereafter, systolic blood pressure increased markedly after 3–4 weeks. Four weeks after the start of DOCA–salt treatment, the systolic blood pressure of vehicle-treated DOCA–salt rats was 213 ± 5 mm Hg, whereas that of the vehicle-treated sham group was 123 ± 2 mm Hg. Treatment with α-lipoic acid at 10 mg/kg tended to attenuate the development of hypertension, but no significant difference was observed between vehicle- and 10 mg/kg α-lipoic acid-treated DOCA–salt groups. Daily administration of α-lipoic acid at 100 mg/kg for 2 weeks almost completely abolished any further increases in blood pressure. Systolic blood pressure after 4 weeks for the 100 mg/kg α-lipoic acid-treated group was 157 ± 4 mm Hg, the value being similar to that observed at the start of α-lipoic acid administration (155 ± 3 mm Hg at 2 weeks). Treatment of sham-operated rats with α-lipoic acid at 100 mg/kg did not affect the blood pressure during the experimental period.

3.2. Effects of treatment with α-lipoic acid on body, aorta and kidney weights of DOCA–salt hypertensive rats

At the end of the experiment (at 4 weeks), the gain in body weight in vehicle-treated DOCA–salt rats was less than that in vehicle-treated sham rats (Table 1). Treatment with α-lipoic acid led to the recovery of losses. When left kidney weight was corrected by body weight, there was significant increase in kidney weight-to-body weight ratio in vehicle-treated DOCA–salt rats. Aortic weight corrected by length also showed a significant increase by DOCA–salt treatment. These increments were significantly suppressed by treatment with α-lipoic acid at 100 mg/kg but not at 10 mg/kg. Qualitatively similar findings were obtained between vehicle- and 100 mg/kg α-lipoic acid-treated sham groups.

3.3. Effects of treatment with α-lipoic acid on blood and urinary parameters of DOCA–salt hypertensive rats

Table 2 summarized renal functional parameters at the end of the experimental period. Significant decrease in creatinine clearance was observed in vehicle-treated DOCA–salt hypertensive rats. On the other hand, the levels of urinary excretion of protein and *N*-acetyl-β-glucosaminidase, and fractional excretion of sodium in vehicle-treated DOCA–salt hypertensive rats were markedly elevated compared with findings in vehicle-treated sham rats. These functional changes were ameliorated by treatment with α-lipoic acid dose-dependently. α-Lipoic acid at

Table 1
Comparative data on body, kidney, and aorta weights in sham-operated and deoxycorticosterone acetate (DOCA)–salt rats treated with vehicle or α-lipoic acid

Parameter	Sham		DOCA–salt		
	Vehicle	LA 100 mg/kg	Vehicle	LA 10 mg/kg	LA 100 mg/kg
Body weight (g)	343 ± 8	330 ± 10	279 ± 9[a]	312 ± 6[b]	310 ± 8[b]
Kidney weight (g/BW kg)	4.4 ± 0.04	4.6 ± 0.01	10.1 ± 0.61[a]	8.8 ± 1.01	7.4 ± 0.13[b]
Aorta weight (mg/cm)	10.3 ± 0.3	11.3 ± 0.2	13.2 ± 0.8[a]	12.0 ± 0.4	11.1 ± 0.4[c]

Each value represents the mean ± S.E.M ($n = 6$). LA: α-lipoic acid; BW: body weight.
[a] $P < 0.01$, compared with vehicle-treated sham rats.
[b] $P < 0.05$ compared with vehicle-treated DOCA–salt rats.
[c] $P < 0.01$ compared with vehicle-treated DOCA–salt rats.

Table 2
Comparative data on blood and urinary parameters in sham-operated and deoxycorticosterone acetate (DOCA)–salt rats treated with vehicle or α-lipoic acid

Parameter	Sham		DOCA–salt		
	Vehicle	LA 100 mg/kg	Vehicle	LA 10 mg/kg	LA 100 mg/kg
Ccr (ml/min/kg BW)	5.67 ± 0.27	5.68 ± 0.51	3.68 ± 0.49[a]	4.03 ± 0.44	6.20 ± 0.56[b]
$U_{protein}V$ (mg/min/kg BW)	0.07 ± 0.01	0.09 ± 0.01	1.81 ± 0.56[c]	0.74 ± 0.26[d]	0.15 ± 0.03[b]
$U_{NAG}V$ (mU/min/kg BW)	0.47 ± 0.28	0.26 ± 0.05	7.68 ± 2.01[c]	4.12 ± 1.73	4.56 ± 0.86
FE_{Na} (%)	0.56 ± 0.06	0.31 ± 0.05	16.1 ± 4.56[c]	7.70 ± 0.83	5.62 ± 0.22[b]

Each value represents the mean ± S.E.M ($n = 6$). LA: α-lipoic acid; BW: body weight; Ccr: creatinine clearance; $U_{protein}V$: urinary excretion of protein; $U_{NAG}V$: urinary excretion of *N*-acetyl-β-glucosaminidase; FE_{Na}: fractional excretion of sodium.
[a] $P < 0.05$ compared with vehicle-treated sham rats.
[b] $P < 0.01$ compared with vehicle-treated DOCA–salt rats.
[c] $P < 0.01$ compared with vehicle-treated sham rats.
[d] $P < 0.05$ compared with vehicle-treated DOCA–salt rats.

100 mg/kg led to greater improvement in functional parameters, achieving statistical significance, except for urinary excretion of *N*-acetyl-β-glucosaminidase. Treatment of sham-operated rats with 100 mg/kg α-lipoic acid did not affect all the renal function parameters.

3.4. Effects of treatment with α-lipoic acid on histological renal damage in DOCA–salt hypertensive rats

Fig. 2 shows typical examples in renal tissues of sham-operated and DOCA–salt rats. Histological examination of the kidney in vehicle-treated DOCA–salt rats revealed lesions characterized by fibrinoid-like necrosis in glomeruli, thickening of small arteries, tubular dilatation and proteinaceous casts in tubuli. Treatment with α-lipoic acid at 100 mg/kg clearly reduced such damage.

3.5. Effects of treatment with α-lipoic acid on vascular hypertrophy of DOCA–salt hypertensive rats

Fig. 3 shows examples of representative cross-sections of the aorta obtained from one animal of each group. An

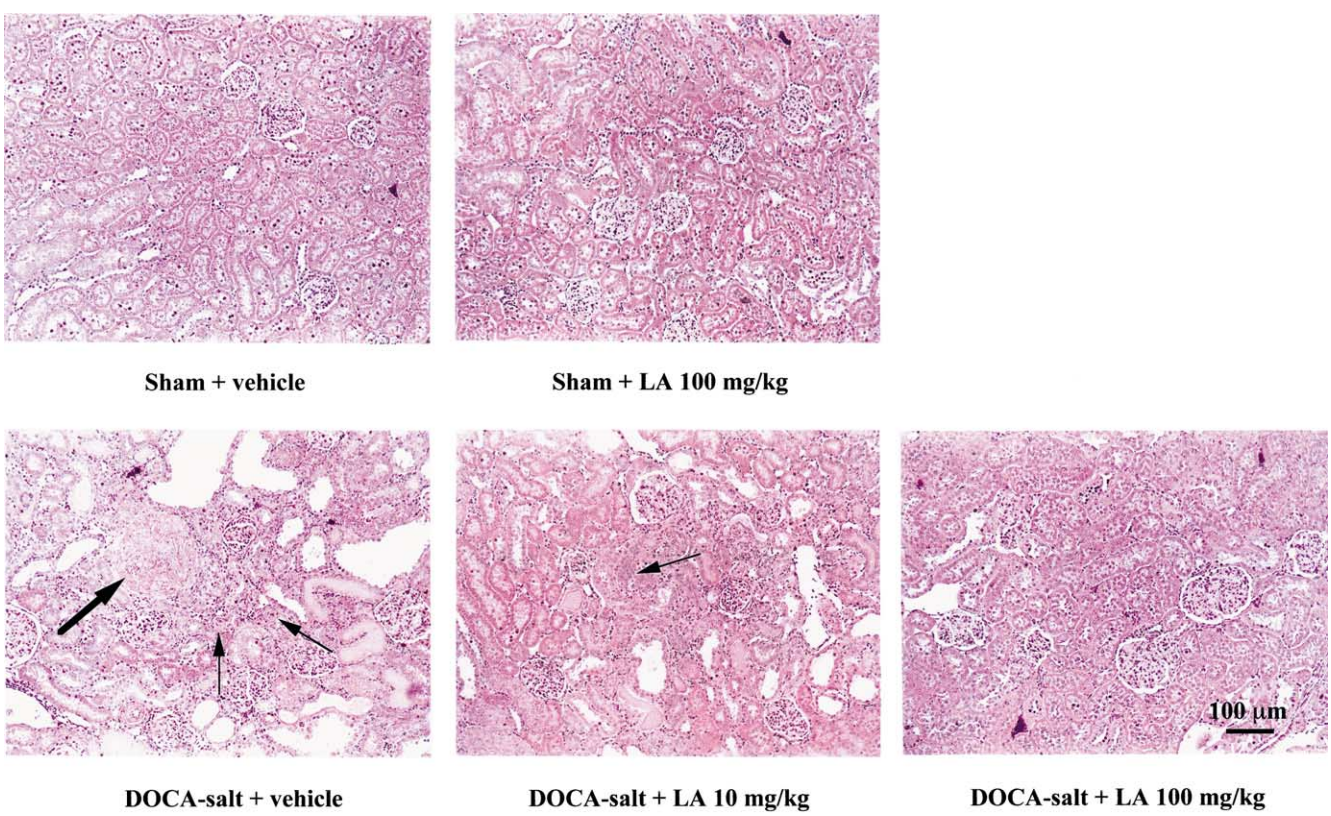

Fig. 2. Representative light micrographs of renal tissues obtained from sham-operated and deoxycorticosterone acetate (DOCA)–salt rats treated with vehicle or α-lipoic acid (LA). Thick arrow indicates fibrinoid-like necrosis in glomeruli. Thin arrows indicate thickening of small arteries.

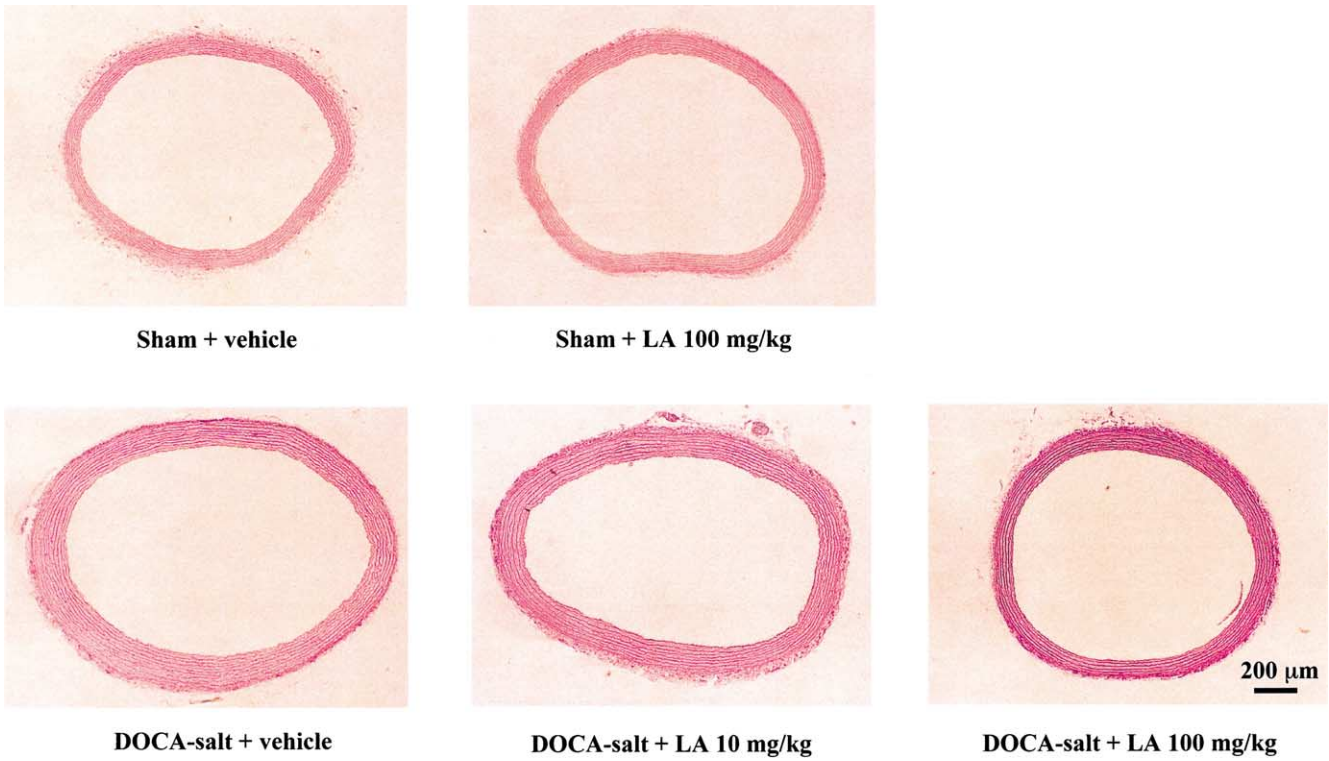

Fig. 3. Representative light micrographs showing cross-sections of thoracic aortas obtained from sham-operated and deoxycorticosterone acetate (DOCA)–salt rats treated with vehicle or α-lipoic acid (LA).

increase in vascular medial thickness (wall thickness), a characteristic finding for hypertensive arterial hypertrophy, was evident in vehicle-treated DOCA–salt rats. This vascular change was markedly suppressed by treatment with α-lipoic acid at 100 mg/kg. Fig. 4 shows a significant increase in media cross-sectional area (wall area) of vehicle-treated DOCA–salt rats (0.77 ± 0.03 mm^2) compared with vehicle-treated sham rats (0.51 ± 0.02 mm^2). Although 10 mg/kg α-lipoic acid had no effect on this parameter of vascular hypertrophy (0.76 ± 0.02 mm^2), 100 mg/kg α-lipoic acid decreased the parameter and values (0.57 ± 0.03 mm^2) approximated to those obtained in vehicle-treated sham rats. Wall area was not significantly different between vehicle- and 100 mg/kg α-lipoic acid-treated sham rats (0.59 ± 0.03 mm^2).

3.6. Effects of treatment with α-lipoic acid on endothelin-1 contents in the aorta and kidney of DOCA–salt hypertensive rats

As shown in Fig. 5, a marked increase in aortic endothelin-1 content was observed in vehicle-treated

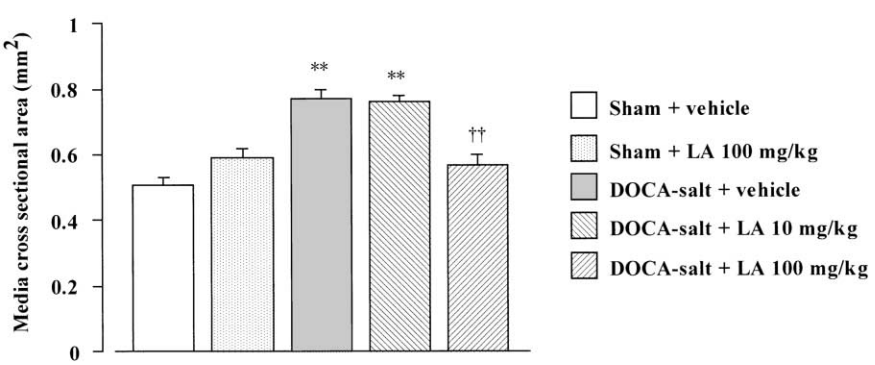

Fig. 4. Effect of α-lipoic acid (LA) on cross-sectional area of aortic media in sham-operated and deoxycorticosterone acetate (DOCA)–salt rats treated with vehicle or α-lipoic acid. Each column and bar represents mean ± S.E.M ($n = 6$). $^{**}P < 0.01$ compared with sham-operated rats. $^{††}P < 0.01$ compared with vehicle-treated DOCA–salt rats.

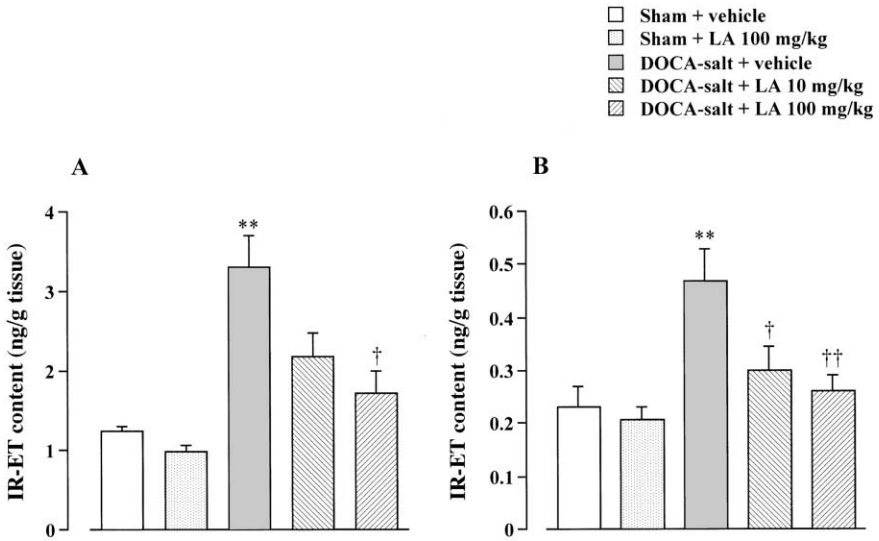

Fig. 5. Effect of α-lipoic acid (LA) on aortic (A) and renal (B) immunoreactive endothelin (IR-ET) content in sham-operated and deoxycorticosterone acetate (DOCA)–salt rats treated with vehicle or α-lipoic acid. Each column and bar represents mean ± S.E.M ($n = 6$). $^{**}P < 0.01$ compared with sham-operated rats. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ compared with vehicle-treated DOCA–salt rats.

DOCA–salt hypertensive rats (3.30 ± 0.44 ng/g tissue), the value being about threefold over the vehicle-treated sham rats (1.24 ± 0.06 ng/g tissue). Treatment of DOCA–salt rats with α-lipoic acid dose-dependently suppressed the increased aortic endothelin-1 content: the higher dose of α-lipoic acid significantly decreased aortic endothelin-1 content in DOCA–salt rats (1.73 ± 0.28 ng/g tissue), but the effect of α-lipoic acid at the lower dose was not statistically significant (2.18 ± 0.31 ng/g tissue). A marked increase in endothelin-1 content was also observed in the kidney of vehicle-treated DOCA–salt hypertensive rats (0.47 ± 0.06 ng/g tissue), the value being about twofold over the vehicle-treated sham rats (0.23 ± 0.05 ng/g tissue). Treatment with α-lipoic acid dose-dependently and significantly suppressed the increase in renal endothelin-1 content induced by DOCA and salt. Endothelin-1 contents in 100 mg/kg α-lipoic acid-treated DOCA–salt rats (0.26 ± 0.03 ng/g tissue) approximated to levels seen in vehicle-treated sham rats. Treatment of sham-operated rats with 100 mg/kg α-lipoic acid did not affect endothelin-1 content in the aorta and kidney.

4. Discussion

The current study showed that α-lipoic acid was capable of preventing the development of hypertension and associated detrimental histopathological changes in aorta and kidney of DOCA–salt hypertensive rats. We concurrently found that the effects of α-lipoic acid were accompanied by decreases in aortic and renal content of endothelin-1, a deleterious mediator in the pathogenesis of DOCA–salt-induced hypertension (Larivière et al., 1993a,b; Fujita et al., 1995, 1996a). Thus, α-lipoic acid appears to suppress the enhanced endothelin-1 production in vascular and renal tissues and the consequent development of hypertension, vascular hypertrophy and renal injury in this model of hypertension.

Recent reports have noted that increased vascular superoxide production and imbalance in liver antioxidant status associated with an increase in lipid peroxidation occur in DOCA–salt-induced hypertension (Somers et al., 2000; Nicod et al., 2000), suggesting that oxidative stress might be closely related to the pathogenesis of this type of hypertension. α-Lipoic acid is known to be as a universal antioxidant. These antioxidant effects are due to direct radical scavenging and metal chelating by both α-lipoic acid and its reduced form dihydrolipoic acid, to the interactions of dihydrolipoic acid with other antioxidants, and to an increase in intracellular glutathione which occurs in cells exposed to exogenous α-lipoic acid. Thus, α-lipoic acid supplied exogenously to cells, tissues, and whole animals exerts powerful antioxidant effects (Packer, 1995). From these findings, it is most likely that α-lipoic acid exhibits its antioxidative effects on DOCA–salt-induced hypertension.

There is accumulating evidence indicating that endothelin-1 plays an important role in the development of hypertension and related tissue injuries such as vascular hypertrophy and renal damage in DOCA–salt-induced hypertension. This view is based on findings that chronic administration of a selective endothelin ET_A receptor antagonist or nonselective endothelin ET_A/ET_B receptor antagonist to DOCA–salt rats suppresses the development of hypertension, vascular hypertrophy and renal injury (Li et al., 1994, 1996, 1998; Schiffrin et al., 1995; Fujita et al., 1996b; Matsumura et al., 1999). It has also been demonstrated that endothelin-1 content and its mRNA expression

are elevated in vascular and renal tissues of DOCA–salt hypertensive rats (Larivière et al., 1993a,b; Fujita et al., 1995, 1996a); however, the mechanism by which endothelin-1 production is enhanced in blood vessels and kidneys in DOCA–salt hypertension are unknown. Interestingly, while there are reports showing that H_2O_2 reduces endothelin-1 release by human umbilical vein endothelial cells (Mitchell et al., 1992) and by rat pulmonary endothelial cells (Michael et al., 1997), one study indicates that incubation with xanthine/xanthine oxidase or H_2O_2 augments endothelin-1 mRNA levels in human renal mesangial cells (Hughes et al., 1996). The reasons for these disparate results have been unclear; however, the latter findings suggest that oxidant stress stimulates endothelin-1 production at a stage of its gene expression. Quehenberger et al. (2000) have recently demonstrated that human endothelin-1 gene has an NF-κB binding site and confirmed that transcription of endothelin-1 is controlled by NF-κB in advanced glycation end product-stimulated cultured endothelial cells. In the activation process of NF-κB, reactive oxygen species is the common signal for a number of stimuli (Sen and Packer, 1996) such as TNF-α, interleukin-1, phorbol myristate acetate and H_2O_2 (Schreck et al., 1991, 1992; Suzuki et al., 1992, 1995). In addition, TNF-α has been demonstrated to increase reactive oxygen species production in mesangial cells (Radeke et al., 1990), as well as augmenting endothelin-1 release and endothelin-1 gene expression in these cells (Kohan, 1992) and in endothelial cells (Marsden and Brenner, 1992). Taken together, it seems that a link exists between oxidative stress-dependent NF-κB activation and induction of endothelin-1 transcription.

The mechanism for suppression of enhanced tissue endothelin-1 production by α-lipoic acid treatment remains speculative. Suzuki et al. (1992) first reported that α-lipoic acid inhibited NF-κB activation in cultured Jurkat T cells. Bierhaus et al. (1997) have noted that α-lipoic acid suppresses advanced glycation end product-induced activation of NF-κB in cultured endothelial cells, and this effect is accompanied by reduced transcription and expression of endothelin-1. We also have observed that α-lipoic acid partially suppresses basal endothelin-1 release and completely suppresses TNF-α-induced endothelin-1 release in cultured endothelial cells (Ohkita et al., unpublished observation). These findings raise the possibility that inhibition of NF-κB activation by α-lipoic acid treatment may be involved in the mechanisms for suppressive effects of α-lipoic acid on increased tissue endothelin-1 contents in DOCA–salt hypertensive rats.

As well as reactive oxygen species, proteasome is known to play a crucial role in the activation of NF-κB: this proteinase is responsible for the activation process of NF-κB at a degradation step of phosphorylated IκB, an inhibitory binding protein of NF-κB (Palombella et al., 1994; Traenckner et al., 1994). We have recently revealed that a proteasome inhibitor, *N*-benzyloxycarbonyl-Ile-Glu(*O*-*t*-Bu)-Ala-leucinal (PSI) has antihypertensive effect on DOCA–salt hypertensive rats (Takaoka et al., 1998) and that PSI attenuates the increased aortic endothelin-1 content in this model of hypertension (Okamoto et al., 1998), thereby suggesting that proteasome plays an important role in the enhanced production of endothelin-1 in blood vessels in DOCA–salt hypertension. Thus, it seems likely that drugs which are capable of inhibiting any step in NF-κB activation may exhibit beneficial effects on the DOCA–salt hypertension. However, there is no circumstantial evidence regarding the change in NF-κB activation during development of hypertension induced by DOCA and salt. Further experiments are required to clarify whether protective effects of α-lipoic acid on the development of hypertension and hypertensive tissue injury in DOCA–salt hypertensive rats are related to suppression of oxidative stress-induced NF-κB activation.

In the present study, we observed that the higher dose of α-lipoic acid reduced the cross-sectional area of aortic media, which represent a degree of vascular hypertrophy, to the level seen in vehicle-treated sham rats and that this treatment reduced systolic blood pressure in DOCA–salt hypertensive rats, but the value was not restored to the level seen in the vehicle-treated sham group; rather, the value was similar to that observed at the start of α-lipoic acid administration. Therefore, the suppressive effect of α-lipoic acid at the higher dose on the vascular hypertrophy is likely to be greater than that on blood pressure elevation in DOCA–salt hypertensive rats. Hypertension, in human and in experimental animals, is often accompanied by vascular hypertrophy (Folkow, 1982). Elevated blood pressure itself has been considered to play a major role in vascular hypertrophy. However, it seems unlikely that in cases of DOCA–salt-induced hypertension, the development of hypertrophy is simply an adaptive response to elevated blood pressure. Indeed, Li et al. (1994), for the first time, observed a blood pressure-independently suppressive effect of nonselective endothelin receptor antagonist bosentan on hypertrophy of mesenteric resistant arteries in this model of hypertension. This observation was then extended and further supported by Schiffrin et al. (1996). We also reported that a marked decrease in aortic hypertrophy was observed in DOCA–salt hypertensive rats treated with the selective endothelin ET_A-receptor antagonist, FR139317 [(*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]carbonyl]amino-4-methyl-pentanoyl]amino-3-[3-(1-methyl-1*H*-indoyl)]propionyl]amino-3-(2-pyridyl) propionic acid] (Fujita et al., 1996b) or ABT-627 [2*R*-(4-methoxyphenyl)-4*S*-(1,3-benzodioxol-5-yl)-1-(*N*,*N*-di(*n*-butyl)aminocarbonyl-methyl)-pyrrolidine-3*R*-carboxylic acid] (Matsumura et al., 1999), and that the decrease in vascular hypertrophy was clear-cut, even though the systolic blood pressure after treatment with each antagonist remained at the hypertensive level. Such observations imply that the endothelin ET_A-receptor antagonist prevents the development of vascular hypertrophy, independently of

its antihypertensive actions. This also suggests that endothelin-1, which has proliferative effects on vascular smooth muscle cells (Dubin et al., 1989; Hirata et al., 1989; Ohlstein et al., 1992), in addition to its vasoconstrictor properties, contributes to the pathogenesis of the vascular hypertrophy in DOCA–salt hypertensive rats. Thus, the effectiveness of α-lipoic acid on vascular hypertrophy induced by DOCA–salt treatment, possibly through the repression of enhanced endothelin-1 production, may also be independent of its antihypertensive effects.

While the present work was in progress, Vasdev et al. (2000) reported that dietary α-lipoic acid supplementation lowers blood pressure in spontaneously hypertensive rats (SHR). In their experiments, SHRs were given a diet supplemented with α-lipoic acid (500 mg/kg feed) for 9 weeks. The treatment of SHRs with α-lipoic acid attenuated not only systolic blood pressure, but also platelet cytosolic free Ca^{2+}, blood glucose and insulin levels, and tissue aldehyde conjugates, all of which were higher in SHRs than in its control Wistar–Kyoto rats. These investigators, however, did not measure tissue endothelin-1 levels in SHRs, and we did not assess the biochemical parameters evaluated by them in DOCA–salt hypertensive rats. It therefore remains obscure regarding the common mechanism for preventive effects of α-lipoic acid on the two types of hypertension. In any case, these findings suggest that α-lipoic acid may have clinical benefits in preventing hypertension and hypertensive tissue injury.

In conclusion, our results indicate that long-term treatment with α-lipoic acid efficiently overcome the development of hypertension, vascular hypertrophy and renal injury in DOCA–salt hypertensive rats, possibly through the suppression of endothelin-1 overproduction. This suggests the possible involvement of oxidative stress in mechanisms underlying enhanced endothelin-1 production in blood vessels and kidneys of DOCA–salt hypertensive rats.

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References

Bierhaus, A., Chevion, S., Chevion, M., Hofmann, M., Quehenberger, P., Illmer, T., Luther, T., Berentshtein, E., Tritschler, H., Müller, M., Wahl, P., Ziegler, R., Nawroth, P.P., 1997. Advanced glycation end product-induced activation of NF-κB is suppressed by α-lipoic acid in cultured endothelial cells. Diabetes 46, 1481–1490.

Deng, L.Y., Day, R., Schiffrin, E.L., 1996. Localization of sites of enhanced expression of endothelin-1 in the kidney of DOCA–salt hypertensive rats. J. Am. Soc. Nephrol. 7, 1158–1164.

Dubin, D., Pratt, R.E., Cooke, J.P., Dzau, V.J., 1989. Endothelin, a potent vasoconstrictor, is a vascular smooth muscle mitogen. J. Vasc. Med. Biol. 1, 150–154.

Folkow, B., 1982. Physiological aspects of primary hypertension. Physiol. Rev. 62, 347–354.

Fujita, K., Matsumura, Y., Kita, S., Miyazaki, Y., Hisaki, K., Takaoka, M., Morimoto, S., 1995. Role of endothelin-1 and the ET_A receptor in the maintenance of deoxycorticosterone acetate–salt-induced hypertension. Br. J. Pharmacol. 114, 925–930.

Fujita, K., Matsumura, Y., Miyazaki, Y., Hashimoto, N., Takaoka, M., Morimoto, S., 1996a. ET_A receptor-mediated role of endothelin in the kidney of DOCA–salt hypertensive rats. Life Sci. 58, PL1–PL7.

Fujita, K., Matsumura, Y., Miyazaki, Y., Takaoka, M., Morimoto, S., 1996b. Effects of endothelin ET_A-receptor antagonist FR139317 on development of hypertension and cardiovascular hypertrophy in deoxycorticosterone acetate–salt hypertensive rats. Jpn. J. Pharmacol. 70, 313–319.

Hexum, T.D., Hoeger, C., River, J.E., Baird, A., Brown, M.R., 1990. Characterization of endothelin secretion by vascular endothelial cells. Biochem. Biophys. Res. Commun. 167, 294–300.

Hirata, Y., Takagi, Y., Fukuda, Y., Marumo, F., 1989. Endothelin is a potent mitogen for vascular smooth muscle cell. Atherosclerosis 78, 225–228.

Hughes, A.K., Stricklett, P., Padilla, E., Kohan, D.E., 1996. Effect of reactive oxygen species on endothelin-1 production by human mesangial cells. Kidney Int. 49, 181–189.

Kohan, D.E., 1992. Production of endothelin-1 by rat mesangial cells: regulation by tumor necrosis factor. J. Lab. Clin. Med. 119, 477–484.

Larivière, R., Thibault, G., Schiffrin, E.L., 1993a. Increased endothelin-1 content in blood vessels of deoxycorticosterone acetate–salt hypertensive but not in spontaneously hypertensive rats. Hypertension 21, 294–300.

Larivière, R., Day, R., Schiffrin, E.L., 1993b. Increased expression of endothelin-1 gene in blood vessels of deoxycorticosterone acetate–salt hypertensive rats. Hypertension 21, 916–920.

Li, J.S., Larivière, R., Schiffrin, E.L., 1994. Effect of a nonselective endothelin antagonist on vascular remodeling in deoxycorticosterone acetate–salt hypertensive rats: evidence for a role of endothelin in vascular hypertrophy. Hypertension 24, 183–188.

Li, J.S., Schurch, W., Schiffrin, E.L., 1996. Renal and vascular effects of chronic endothelin receptor antagonism in malignant hypertensive rats. Am. J. Hypertens. 9, 803–811.

Li, J.-S., Turgeon, A., Schiffrin, E.L., 1998. Effect of chronic treatment with two different ET_A selective endothelin receptor antagonists on blood pressure and small artery structure of deoxycorticosterone acetate (DOCA)–salt hypertensive rats. Am. J. Hypertens. 11, 554–562.

Marsden, P.A., Brenner, B.M., 1992. Transcriptional regulation of the endothelin-1 gene by TNF-α. Am. J. Physiol. 262, C854–C861.

Matsumura, Y., Ikegawa, R., Takaoka, M., Morimoto, S., 1990. Conversion of porcine big endothelin to endothelin by an extract from the porcine aortic endothelial cells. Biochem. Biophys. Res. Commun. 167, 203–210.

Matsumura, Y., Hashimoto, N., Taira, S., Kuro, T., Ohkita, M., Opgenorth, T.J., Takaoka, M., 1999. Different contribution of endothelin-A and endothelin-B receptors in the pathogenesis of deoxycorticosterone acetate–salt-induced hypertension in rats. Hypertension 33, 759–765.

Michael, J.R., Markewitz, B.A., Kohan, D.E., 1997. Oxidant stress regulates basal endothelin-1 production by cultured rat pulmonary endothelial cells. Am. J. Physiol. 273, L768–L774.

Mitchell, M.D., Branch, D.W., Lamarche, S., Dudley, D.J., 1992. The regulation of endothelin production in human umbilical vein endothelial cells: unique inhibitory action of calcium ionophores. J. Clin. Endocrinol. Metab. 75, 665–668.

Nicod, L., Rodriguez, S., Letang, J.M., Viollon, Abadie, C., Jacqueson, A., Berthelot, A., Richert, L., 2000. Antioxidant status, lipid peroxidation, mixed function oxidase and UDP-glucuronyl transferase activities in livers from control and DOCA–salt hypertensive male Sprague Dawley rats. Mol. Cell. Biochem. 203, 33–39.

Ohlstein, E.H., Arleth, A., Bryan, H., Elliott, J.D., Sung, C.P., 1992. The selective endothelin ET_A receptor antagonist BQ123 antagonizes endothelin-1-mediated mitogenesis. Eur. J. Pharmacol. 225, 347–350.

Okamoto, H., Takaoka, M., Ohkita, M., Itoh, M., Nishioka, M., Matsumura, Y., 1998. A proteasome inhibitor lessens the increased aortic endothelin-1 content in deoxycorticosterone acetate–salt hypertensive rats. Eur. J. Pharmacol. 350, R11–R12.

Packer, L., 1998. α-Lipoic acid: a metabolic antioxidant which regulates NF-κB signal transduction and protects against oxidative injury. Drug Metab. Rev. 30, 245–275.

Packer, L., Witt, E.H., Tritschler, H.J., 1995. Alpha-lipoic acid as a biological antioxidant. Free Radical Biol. Med. 19, 227–250.

Palombella, V., Rando, O.J., Goldberg, A.L., Maniatis, T., 1994. The ubiquitin–proteasome pathway is required for processing the NF-κB precursor protein and the activation of NF-κB. Cell 78, 773–785.

Quehenberger, P., Bierhaus, A., Fasching, P., Muellner, C., Klevesath, M., Hong, M., Stier, G., Sattler, M., Schleicher, E., Speiser, W., Nawroth, P.P., 2000. Endothelin-1 transcription is controlled by nuclear factor-κB in AGE-stimulated cultured endothelial cells. Diabetes 49, 1561–1570.

Radeke, H.H., Meier, B., Topley, N., Flöege, J., Habermehl, G.G., Resch, L., 1990. Interleukin 1-α and tumor necrosis factor-α induce oxygen radical production in mesangial cells. Kidney Int. 37, 767–775.

Reed, L.J., 1974. Multienzyme complex. Acc. Chem. Res. 7, 40–46.

Schiffrin, E.L., Sventek, P., Li, J.-S., Turgeon, A., Reudelhuber, T., 1995. Antihypertensive effect of an endothelin receptor antagonist in DOCA–salt spontaneously hypertensive rats. Br. J. Pharmacol. 115, 1377–1381.

Schiffrin, E.L., Larivière, R., Li, J.S., Sventek, P., 1996. Enhanced expression of the endothelin-1 gene in blood vessels of DOCA–salt hypertensive rats: correlation with vascular structure. J. Vasc. Res. 33, 235–248.

Schreck, R., Rieber, P., Baeuerle, P.A., 1991. Reactive oxygen intermediates as apparently widely used messengers in the activation of NF-κB transcription factor and HIV-1. EMBO J. 10, 2247–2258.

Schreck, R., Meier, B., Männel, D.N., Drüge, W., Baeuerle, P.A., 1992. Dithiocarbamates as potent inhibitors of nuclear factor κB activation in intact cells. J. Exp. Med. 175, 1181–1194.

Sen, C.K., Packer, L., 1996. Antioxidant and redox regulation of gene transcription. FASEB J. 10, 709–720.

Somers, M.L., Mavromatis, K., Galis, Z.S., Harrison, D.G., 2000. Vascular superoxide production and vasomotor function in hypertension induced by deoxycorticosterone acetate–salt. Circulation 101, 1722–1728.

Suzuki, Y.J., Aggarwal, B.B., Packer, L., 1992. α-Lipoic acid is a potent inhibitor of NF-κB activation in human T cells. Biochem. Biophys. Res. Commun. 189, 1709–1715.

Suzuki, Y.J., Mizuno, M., Tritschler, H.J., Packer, L., 1995. Redox regulation of NF-κB DNA binding activity by dihydrolipoate. Biochem. Mol. Biol. Int. 36, 241–246.

Takaoka, M., Okamoto, H., Ito, M., Nishioka, M., Kita, S., Matsumura, Y., 1998. Antihypertensive effect of a proteasome inhibitor in DOCA–salt hypertensive rats. Life Sci. 63, PL65–PL70.

Traenckner, E.B.-M., Wilk, S., Baeuerle, P.A., 1994. A proteasome inhibitor prevents activation of NF-κB and stabilizes a newly phosphorylated form of IκB-α that is still bound to NF-κB. EMBO J. 13, 5433–5441.

Vasdev, S., Ford, C.A., Parai, S., Longerich, L., Gadag, V., 2000. Dietary α-lipoic acid supplementation lowers blood pressure in spontaneously hypertensive rats. J. Hypertens. 18, 567–573.