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A proteasome inhibitor lessens the increased aortic endothelin-1 content in deoxycorticosterone acetate-salt hypertensive rats

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Abstract

Deoxycorticosterone acetate (DOCA)-salt-treated rats developed marked hypertension after 4 weeks with an increase in aortic endothelin-1. Treatment of DOCA-salt hypertensive rats with a proteasome inhibitor, *N*-benzyloxycarbonyl-Ile-Glu(*O*-*t*-Bu)-Ala-leucinal, significantly reduced the elevation in systolic blood pressure and the effect was accompanied by a decrease in aortic endothelin-1 content. Thus, a proteasome-dependent proteolytic pathway appears to play an important role in the enhanced production of endothelin-1 in blood vessels and the consequent increase in blood pressure in this model of hypertension.

Keywords: Proteasome inhibitor; Endothelin-1; Deoxycorticosterone acetate (DOCA)-salt hypertension

Endothelin-1 is a potent vasoconstrictor peptide isolated from vascular endothelial cells (Yanagisawa et al., 1988). Endothelin-1 has been implicated in the pathogenesis of hypertension in humans and in experimental animals. We reported that the vascular endothelin-1 content is increased in deoxycorticosterone acetate (DOCA)-salt hypertensive rats, indicating that endothelin-1 contributes to the development and maintenance of DOCA-salt-induced hypertension (Fujita et al., 1995). The mechanisms by which endothelin-1 production is enhanced in blood vessels of DOCA-salt hypertensive rats are obscure. Corder et al. (1997) recently showed that proteasome inhibitors blocked tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β)-stimulated endothelin-1 synthesis in bovine aortic endothelial cells. This led us to speculate that proteasome may represent a potent target for endothelin-1-related cardiovascular diseases and that proteasome inhibitors could be useful for the attenuation of the enhanced production of endothelin-1 in blood vessels in vivo. In the present study, we examined whether a proteasome inhibitor, *N*-benzyloxycarbonyl-Ile-Glu(*O*-*t*-Bu)-Ala-leucinal (PSI), would reduce the increased endothelin-1 content in aorta of DOCA-salt hypertensive rats.

Male Sprague–Dawley rats (Japan SLC, Shizuoka) weighing about 190 g were used. DOCA-salt hypertensive rats were prepared as described (Fujita et al., 1995). Briefly, unilaterally nephrectomized rats were treated twice weekly with DOCA suspended in corn oil, administered subcutaneously (15 mg/kg) and 1% NaCl was added to their tap water for drinking. Sham-operated rats (sham group) were unilaterally nephrectomized but were not given DOCA or salt. Two weeks after the start of DOCA-salt treatment, the DOCA-salt rats were randomly divided into two groups (PSI and control groups) and were given PSI (3 mg/kg) or the vehicle (a solution consisting of 35% ethanol, 35% polyethylene glycol 400 and 30% saline) for 2 weeks. PSI or vehicle in a volume of 1 ml/kg was injected subcutaneously every other day. Systolic blood pressure was monitored with a tail cuff and a pneumatic pulse transducer. At the end of the experiment (at 4 weeks), all the rats were exsanguinated, and the thoracic aorta was excised. Endothelin-1 was extracted from the aorta, according to the method of Fujita et al. (1995). A radioimmunoassay for endothelin-1 was carried out as described elsewhere (Matsumura et al., 1990). All values are expressed as means ± S.E.M. For statistical analysis, we used one-way analysis of variance combined with

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Table 1
Effects of DOCA-salt and PSI treatment of rats

	Sham	DOCA-salt	
		Control	PSI
Number	5	5	6
SBP (mmHg)	132 ± 4	197 ± 9^{b}	148 ± 5^{d}
Body weight (g)	368 ± 12	313 ± 18^{b}	347 ± 5
Aorta weight (mg/cm)	11.3 ± 0.5	14.0 ± 1.0^{a}	11.7 ± 0.3^{c}
Aortic ET-1 content (ng/g tissue)	0.46 ± 0.02	2.43 ± 0.28^{b}	0.73 ± 0.09^{d}

Each value represents the mean ± S.E.M. SBP: systolic blood pressure; ET-1: endothelin-1.
[a] $P < 0.05$, [b] $P < 0.01$, compared with the value for the sham group.
[c] $P < 0.05$, [d] $P < 0.01$, compared with the value for the control group.

Duncan's multiple range test for multiple comparisons. Differences were considered significant at $P < 0.05$.

As shown in Table 1, the gain in body weight of the control DOCA-salt rats was smaller than that of sham-operated rats. The body weight gain of PSI-treated DOCA-salt rats was similar to that of sham-operated rats and was greater than that of control rats. The systolic blood pressure and aorta weight of the control group were significantly greater than those of sham group, but the administration of PSI significantly lessened the effects on these alterations. In addition, a marked increase in aortic endothelin-1 content was observed in the control group compared with that in the sham group. A significant attenuation of this increase occurred in the PSI group.

Evidence has accumulated for the view that the proteasome-dependent proteolytic pathway serves important functions in cell cycle control or transcriptional regulation. Reagents inhibiting proteasome activity were used for the purpose. Among these reagents, PSI is a potent and relatively selective inhibitor of the chymotrypsin-like activity of proteasome (Figuereido-Pereira et al., 1994). The use of PSI with cultured cells has shown physiological functions of proteasome (Figuereido-Pereira et al., 1994; Traenckner et al., 1994; Griscavage et al., 1996). We found that the increased aortic endothelin-1 contents in DOCA-salt hypertensive rats were lessened by PSI treatment, with the chymotrypsin-like activity of aortic proteasome in this hypertensive rats being inhibited by about 30% (Takaoka et al., unpublished observation). The mechanism for the enhancement of the production of vascular endothelin-1 via a proteasome-dependent proteolytic pathway may be related to any of the different stimuli that increase production of vascular endothelin-1. The stimuli include vasoactive substances and cytokines. A recent observation that proteasome inhibitors block TNF-α and TGF-β-stimulated endothelin-1 synthesis in bovine aortic endothelial cells (Corder et al., 1997), suggests that at least the two cytokines enhance the endothelin-1 production in endothelial cells via the proteasome-dependent proteolytic pathway. It is also of interest that aortic TGF-β mRNA levels are increased in DOCA-salt rats (Sarzani et al., 1989).

In conclusion, the present study demonstrated that proteasome is responsible for the enhanced production of endothelin-1 in the aorta of DOCA-salt hypertensive rats. Together with previous data showing an important role of vascular endothelin-1 in DOCA-salt-induced hypertension (Fujita et al., 1995), the present findings suggest the possible involvement of a vascular proteasome-dependent proteolytic pathway in the mechanisms underlying elevated blood pressure in this model of hypertension.

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References

Corder, R., Hibbert, T., Khan, N., Barker, S., Wood, E., Lees, D., 1997. Proteasome inhibitors block tumor necrosis factor-a and transforming growth factor-b-stimulated endothelin-1 synthesis in bovine aortic endothelial cells. Br. J. Pharmacol. 120 (Suppl.), 316.

Figuereido-Pereira, M.E., Berg, K.A., Wilk, S., 1994. A new inhibitor of the chymotrypsin-like activity in the multicatalytic proteasome complex (20S proteasome) induces accumulation of ubiquitin–protein conjugates in a neuronal cell. J. Neurochem. 63, 1578–1581.

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

Griscavage, J.S., Wilk, S., Ignarro, L.J., 1996. Inhibitors of the proteasome pathway interfere with induction of nitric oxide synthase in macrophages by blocking activation of transcription factor NF-κB. Proc. Natl. Acad. Sci. USA 93, 3308–3312.

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.

Sarzani, R., Brecher, P., Chobanian, A.V., 1989. Growth factor expression in aorta of normotensive and hypertensive rats. J. Clin. Invest. 83, 1404–1408.

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.

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K., Masaki, T., 1988. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332, 411–415.