Involvement of Proteasome in Endothelin-1 Production in Cultured Vascular Endothelial Cells

Mamoru Ohkita, Masanori Takaoka, Yutaka Kobayashi, Eriko Itoh, Hiroko Uemachi and Yasuo Matsumura*

Department of Pharmacology, Osaka University of Pharmaceutical Sciences, Nasahara, Takatsuki, Osaka 569-1094, Japan

Received October 15, 2001 Accepted November 20, 2001

ABSTRACT—We examined whether the proteasome could regulate endothelin (ET)-1 production in vascular endothelial cells (ECs). A proteasome inhibitor N-benzyloxycarbonyl-Ile-Glu (O-t-Bu)-Ala-leucinal (PSI) significantly decreased ET-1 release from ECs by about 25% of the basal release. PSI also suppressed tumor necrosis factor (TNF)-α-induced ET-1 release from ECs in a dose-dependent manner. Similar inhibitory effects were observed using another proteasome inhibitor lactacystin, whereas a calpain inhibitor calpeptin had no apparent effect on ET-1 release. Furthermore, PSI significantly attenuated prepro ET-1 mRNA expression under basal and TNF-α-stimulated conditions. Electrophoretic mobility shift assay showed that proteasome inhibitors diminished TNF-α-stimulated nuclear factor-kappa B (NF-κB) activation in ECs. Pretreatment with antioxidants, pyrrolidine dithiocarbamate and α-lipoic acid, both of which are known to be suppressors of NF-κB activation, effectively attenuated basal and TNF-α-induced ET-1 release. Thus, a proteasome-dependent proteolytic pathway is at least partly involved in ET-1 production under basal conditions, and this proteolytic pathway seems to have a crucial role in ET-1 production enhanced by TNF-α. The reduction of NF-κB activation may be involved in the mechanisms for suppressive effects of proteasome inhibitors on ET-1 gene transcription and the consequent decrease in ET-1 mRNA expression and ET-1 release.

Keywords: Endothelin-1, Endothelial cell, Proteasome, Tumor necrosis factor-α, Nuclear factor-kappa B

Endothelin (ET)-1 is a potent vasoconstrictive peptide purified from the supernatant of cultured porcine aortic endothelial cells (ECs) (1). ET-1 biosynthesis and release appear to be regulated at the transcriptional level because ET-1 release from ECs is constitutive. Several studies have indicated that various substances such as thrombin, angiotensin II, transforming growth factor-β1 and tumor necrosis factor (TNF)-α stimulate ET-1 gene expression in ECs by DNA binding of transcription factors such as activator protein-1 and nuclear factor-1 (2).

The proteasome is a multi-subunit protease complex present in the cytoplasm and nucleus as both 20S (700 kDa) and 26S (200 kDa) forms. The 20S proteasome is essential in the ubiquitin-dependent proteolytic pathway, where it functions as the proteolytic core of the 26S proteasome complex that degrades ubiquitin-conjugated proteins (3). This nonlysosomal proteolytic pathway is vital for numerous regulatory proteins which control cell cycle progression, apoptosis, angiogenesis and antigen presentation (4, 5). In addition, the proteasome-dependent proteolytic pathway is known to be involved in the activation process of a transcription factor, nuclear factor-kappa B (NF-κB) (6, 7).

Reagents that can inhibit proteasome activity have been developed and used to elucidate the roles of proteasome in diverse biological processes (3). We recently found that a proteasome inhibitor N-benzyloxycarbonyl-Ile-Glu (O-t-Bu)-Ala-leucinal (called PSI) exhibits antihypertensive effects in deoxycorticosterone acetate (DOCA)-salt hypertensive rats (8), in which ET-1 contributes to the development and maintenance of hypertension (9). In addition, one interesting observation was that PSI markedly suppressed the increased aortic ET-1 content in DOCA-salt hypertensive rats (10). These findings suggest that a proteasome-dependent proteolytic pathway is possibly involved in the enhanced production of ET-1 in blood vessels in this model of hypertension. However, it remains unknown whether this proteolytic pathway is directly responsible for the ET-1 production.

To evaluate the involvement of a proteasome-dependent proteolytic pathway in ET-1 production, we have now
investigated the effect of proteasome inhibition on basal and TNF-α-induced ET-1 production in cultured porcine aortic ECs and whether the effect would be accompanied by a suppression of NF-κB activation.

MATERIALS AND METHODS

Materials
Recombinant human TNF-α was purchased from Pepro Tech, Inc. (Rocky Hill, NJ, USA). Pyrrolidine dithiocarbamate (PDTC) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). PSI was obtained from Peptide Institute, Inc. (Osaka). α-Lipoic acid (α-LA) was purchased from Nacalai Tesque, Inc. (Kyoto). Lactacystin was obtained from Kyowa Medex Co., Ltd. (Tokyo). Calpeptin was purchased from Wako Pure Chemical Industries, Ltd. (Osaka). [125I]ET-1, [α-32P]dCTP and [γ-32P]ATP were obtained from Amersham Pharmacia Biotech UK Ltd. (Little Chalfont, Buckinghamshire, UK). The culture reagents were purchased from Life Technologies Inc. (Grand Island, NY, USA). All other materials used were commercial products of the highest grade available.

Cell culture
Porcine aortic ECs were isolated and grown on gelatin-coated Petri dishes or plate in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal calf serum, 100 U/ml of penicillin and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 95% air and 5% CO₂ as described (11). ECs were identified by the typical phase contrast “cobblestone” morphology and by immunofluorescence to Factor VIII-related antigen. All cell cultures were used from passages 6 to 12. For all experiments, ECs were grown to confluence and then made quiescent by incubation with serum-free DMEM containing 0.01% heat-inactivated bovine serum albumin (Sigma Chemical Co.) for 12 h.

Radioimmunoassay (RIA) for determination of ET-1
The amount of ET-1 secretion from ECs was determined using RIA. Quiescent ECs were incubated for various times with or without chemical treatments. At the end of incubation, the culture medium was aspirated off, boiled for 5 min and then centrifuged at 8,000 x g for 10 min. The resulting supernatant was stored at −30°C until assay for RIA.

The RIA for ET-1 was performed as described (11). ET-1 antiserum that does not cross-react with big ET-1 (12) was kindly provided by Dr. M.R. Brown, University of California (San Diego, CA, USA). The standard buffer of RIA was 50 mM sodium phosphate buffer, pH 7.4, containing 50 mM NaCl, 0.1% Triton X-100 and 1 mM EDTA. A mixture of 0.1 ml each of standard ET-1 or sample, assay buffer and antiserum (final dilution of 1:12,000) was incubated at 4°C for 24 h, followed by the addition of 0.1 ml of [125I]ET-1 (approximately 10,000 cpm). After 48-h incubation at 4°C, 0.1 ml of goat anti-rabbit γ-globulin (ICN Pharmaceuticals Inc., Costa Mesa, CA, USA) and 0.3 ml of 16.5% polyethylene glycol were added to the mixture and the preparation was further incubated at 4°C for 4 h. Bound and free ligands were separated by centrifugation at 3,000 rpm for 30 min. The bound radioactivity was counted in a gamma spectrometer (Model ARC-301; Aloka Co., Ltd., Tokyo).

Northern blot analysis
All procedures were done, according to the method described elsewhere (13). Total RNA was isolated using the acid guanidium thiocyanate-phenol-chloroform extraction method. The isolated total RNA (5 μg per lane) was subjected to electrophoresis on a 1.1% agarose gel containing formaldehyde and transferred to Hybond-XL membrane (Amersham Pharmacia Biotech UK Ltd.). This membrane was prehybridized for 1 h at 68°C in PerfectHyb hybridization solution (Toyobo Inc., Osaka) and then hybridized with porcine prepro ET-1 cDNA probe (a gift from Dr. K. Goto, University of Tsukuba) and GAPDH cDNA probe (Clontech Laboratories, Inc., Palo Alto, CA, USA) labeled with [α-³²P]dCTP, using the Random Primer DNA Labeling Kit (Takara Shuzo Co., Ltd., Kyoto). After hybridization, the membrane was washed two times for 5 min followed by two washes for 15 min at 68°C in 2 x standard saline citrate/0.1% SDS (standard saline citrate contains 150 mM NaCl and 15 mM sodium citrate, pH 7.0). Autoradiography was done by exposing the membrane to Kodak X-Omat AR film (Eastman Kodak Co., Rochester, NY, USA) at −80°C with intensifying screens. The autoradiograms of ET-1 were quantified by densitometric analysis, and signals of ET-1 mRNA were normalized for each sample, with respect to density of the corresponding signal for GAPDH mRNA.

Preparation of nuclear extracts and electrophoretic mobility shift assay (EMSA)
Nuclear extracts were prepared from ECs using the method of Schreiber et al. (14). Briefly, ECs were washed with ice-cold phosphate buffer saline and pelleted. Then the cell pellet was resuspended in hypotonic buffer (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol and 0.5 mM phenylmethylsulfonyl fluoride); incubated for 15 min on ice; and then lysed by the addition of 0.6% Nonidet P-40, followed by vigorous vortexing for 15 s. The nuclei were pelleted and resuspended in extraction buffer (20 mM HEPES, pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride and 10%
glycerol), and the tube was vigorously shaken for 15 min at 4°C. The nuclear extracts were then centrifuged and stored at −80°C.

The nuclear extracts (2 μg protein) were used for EMSA. Double-stranded oligonucleotide containing the most common NF-κB consensus binding site 5'-AGT TGA GGG GAC TTT CCC AGG C-3' (Promega Corporation, Madison, WI, USA) was end-labeled with [γ-32P]-ATP, using T4 polynucleotide kinase (Promega Corporation). The binding reaction was performed for 20 min at 25°C in a total volume of 10 μl of binding buffer that contained 10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.5 mM EDTA, 1 mM MgCl2, 0.5 mM DTT, 4% glycerol and 0.5 μg of double stranded poly (dI-dC). DNA-protein complexes were resolved on 6% nondenaturing polyacrylamide gels at 100 V for 2 h in 0.5× Tris borate-EDTA (1× Tris borate-EDTA which contains 90 mM Tris-borate, pH 8.3, 2 mM EDTA) at 4°C. After electrophoresis, gels were covered with plastic wrap and exposed to Kodak X-Omat AR film at −80°C. The protein-DNA complexes were visualized by autoradiography. To demonstrate the specificity of DNA protein binding, the reactions were performed in the presence of a nonlabeled consensus oligonucleotide competitor. The supershift assay was done using 2 μg of polyclonal IgG against p50 or p65 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA).

**Statistical analyses**

All values are expressed as the mean ± S.E.M. For statistical analysis, we used the unpaired Student’s t-test for two-group comparisons and one-way analysis of variance followed by Dunnett’s or Bonferroni’s multiple comparison tests. Differences were considered statistically significant at P<0.05.

---

**Fig. 1.** Effects of TNF-α and PSI on ET-1 release from cultured porcine aortic ECs. A, time course of the effect of TNF-α on ET-1 release from cultured porcine aortic ECs. Quiescent ECs were incubated for 6–24 h, with or without TNF-α (10 ng/ml). After incubation for each indicated time, the amount of ET-1 in the culture medium was measured using RIA. Each point and bar represents the mean ± S.E.M. (n = 6). **P<0.01, compared with the control value. B, top: effects of PSI on ET-1 release from cultured porcine aortic ECs. Quiescent ECs were treated with TNF-α (10 ng/ml) in the absence or presence of PSI (1 μM). After these treatments for 12 h, the amount of ET-1 in the culture medium was measured using RIA. Each column and bar represents the mean ± S.E.M. (n = 9). **P<0.01, compared with the control value in the absence of PSI. ‡P<0.01, compared with the value of TNF-α in the absence of PSI. Bottom: the percentage of inhibition of ET-1 release in the case of treatment with PSI. ##P<0.01, compared with the control value.
RESULTS

Effects of proteasome inhibition on ET-1 release from ECs

To elucidate the involvement of proteasome in ET-1 production, we first examined the effects of PSI on basal and TNF-α-induced ET-1 release from ECs. The amount of ET-1 release from ECs increased in a linear fashion up to 24 h with or without TNF-α (10 ng/ml). TNF-α increased ET-1 release to about 1.5-fold over the basal level at each indicated time (Fig. 1A). When ECs were incubated with 1 μM PSI for 12 h, the ET-1 release was decreased by 25.5% of basal release (Fig. 1B). TNF-α-induced enhancement of ET-1 release was also reduced by 1 μM PSI, which elicited a 37.3% decrease. Figure 2 shows dose-dependent effects of PSI on ET-1 release from ECs incubated for 12 h, under basal and TNF-α-stimulated conditions. At a concentration of 0.1 μM PSI, ET-1 release was significantly decreased by 24.8% from the basal level. No further decrease in ET-1 release was observed when ECs were exposed to higher concentrations of PSI. PSI produced a dose-dependent decreasing effect on TNF-α-stimulated ET-1 release and showed an almost complete inhibition of this ET-1 release at concentrations of over 1 μM. We next asked if another proteasome inhibitor lactacystin would suppress the ET-1 release (Table 1). Lactacystin also produced dose-related decrease in ET-1 release, with or without TNF-α. In contrast, the calpain inhibitor calpeptin had no significant effect on basal and TNF-α-induced ET-1 release.

Effects of proteasome inhibition on prepro ET-1 mRNA expression in ECs

Effects of PSI on prepro ET-1 mRNA expression were examined using Northern blot analysis. When ECs were incubated in the presence of TNF-α (10 ng/ml), prepro ET-1 mRNA expression rapidly increased as early as 15 min (normalized prepro ET-1 mRNA in the TNF-α-stimulated condition was about 1.3-fold of the control value). PSI at 1 μM had no significant effect on prepro ET-1 mRNA expression with or without TNF-α treatment at 1 h. Thereafter, however, PSI exerted an inhibitory effect on prepro ET-1 mRNA expression. After incubation for 4 h, PSI decreased the mRNA expression by 31.6% and 44.7% under basal and TNF-α-stimulated conditions, respectively (Fig. 3).

Table 1. Effects of proteasome and calpain inhibitors on ET-1 release from cultured porcine aortic ECs

| Treatment   | % Inhibition | 1 μM | 10 μM |
|-------------|--------------|------|-------|
|             | basal        | TNF-α-stimulated | basal | TNF-α-stimulated |
| PSI         | 27.82 ± 3.57** | 43.78 ± 1.48** | 27.17 ± 1.99** | 47.75 ± 2.43** |
| Lactacystin | 1.99 ± 0.71  | 16.52 ± 1.48** | 11.83 ± 1.24** | 35.27 ± 1.66** |
| Calpeptin   | −4.90 ± 1.68 | −0.64 ± 2.63 | 0.47 ± 1.51 | 5.63 ± 1.41 |

Results are expressed as the % inhibition derived from each buffer control. Each value is expressed as the mean ± S.E.M. (n = 9). **P<0.01, compared with control. †P<0.01, compared with TNF-α.
Effects of proteasome inhibition on NF-κB activation in ECs

We further examined the effects of proteasome inhibitors on basal and TNF-α-induced NF-κB activation in ECs using EMSA, because there is considerable evidence that TNF-α causes various biological and pathological actions, mainly through the NF-κB activation in ECs (15). TNF-α (10 ng/ml) induced NF-κB activation within 5 min and the distinct activation of NF-κB remained constant for at least 240 min. The TNF-α-induced NF-κB protein DNA complex was competed by the excessive unlabeled NF-κB consensus oligonucleotide (Fig. 4A). As shown in Fig. 4B,
PSI attenuated the TNF-α-induced NF-κB activation, in a dose- and time-dependent manner. PSI also suppressed the NF-κB activation under the basal condition. Although the results shown in Fig. 4B (lanes 2 and 5) are not clear, the suppressive effect of PSI was detectable when gels were exposed for longer periods (data not shown). For characterization of the NF-κB subunits, specific antibodies for p50 and p65 subunits were used. The TNF-α-induced NF-κB protein DNA complex supershifted by anti-p50 or by anti-p65. Addition of both anti-p50 and anti-p65 supershifted completely the NF-κB protein DNA complex (Fig. 4C). Lactacystin also attenuated TNF-α-induced NF-κB activation, albeit this attenuation being somewhat weak compared with that of PSI (Fig. 5).

Effects of antioxidants on ET-1 release from ECs
To further clarify whether activation of NF-κB is responsible for the TNF-α-stimulated ET-1 production, we examined the effect of antioxidants, such as PDTC and α-LA, on basal and TNF-α-induced ET-1 release from ECs. The antioxidants we used are known to suppress the activation of NF-κB (16, 17). As shown in Fig. 6, PDTC (0.1 mM) and α-LA (2 mM) reduced ET-1 release by 38.4% and 20.6% from the control value of 86.2 ± 2.3 fmol/10^5 cells, respectively. TNF-α-induced ET-1 release (132.4 ± 3.5 fmol/10^5 cells) was more efficiently suppressed by PDTC and α-LA (decreases in ET-1 release were 36.0% and 44.7%, respectively).

DISCUSSION
The present study showed that proteasome inhibitors such as PSI and lactacystin partially suppressed basal ET-1 release and completely suppressed TNF-α-induced ET-1 release from cultured ECs. We concurrently found that these effects were accompanied by reduced ET-1 mRNA expression and NF-κB activation.

Various substrate-related peptide aldehyde derivatives including PSI have been developed for the purpose of inhibiting proteasome (3). The use of PSI in animals and cell culture models has revealed the physiological functions and pathophysiological roles of proteasome (7, 8, 10, 18–20). PSI is recognized as a potent and a cell-permeable inhibitor of proteasome but it does have weak calpain-inhibiting activity (18). One may point out that the suppressive effect of PSI on ET-1 release from ECs is at least
partly due to its inhibitory action on calpain. Thus, we used a potent calpain inhibitor calpeptin to evaluate the involvement of calpain inhibition in the PSI’s action. However, calpeptin at 1 and 10 μM, concentrations which inhibit completely a calpain activity (21), had little effect on ET-1 release. Therefore, it is reasonable to consider that the preventive effect of PSI on ET-1 release from ECs is not due to the inhibition of calpain but must result from the inhibition of proteasome.

Moreover, we used lactacystin, which is known to be a specific proteasome inhibitor that does not affect other proteinases examined so far (22), to confirm the involvement of proteasome in ET-1 release from ECs and obtained evidence in support of the findings from experiments with PSI. Lactacystin produced dose-related decreases in ET-1 release, as well as PSI. However, the suppressive effect of lactacystin was less potent than that of PSI at the same concentrations. The reason for this different efficacy between PSI and lactacystin is unclear. Lactacystin is a water-soluble microbial metabolite, whereas PSI is a peptide aldehyde derivative and therefore is likely to cross plasma membranes in living mammalian cells (3, 18). Dick et al. (23) found that lactacystin hydrolyzes in aqueous solution through a mechanism that involves transient formation of the intermediate clasto-lactacystin β-lactone and that lactacystin, per se, is not a proteasome inhibitor and, rather, the intermediate β-lactone is the sole inhibitory species. They also demonstrated that lactacystin undergoes spontaneous hydrolysis to yield the inactive analog, clasto-lactacystin dihydroxy acid, in aqueous solution at alkaline pH. Taken together, one possible explanation is that intracellular concentration of lactacystin and/or its intermediate β-lactone may not reach a level expected to inhibit proteasome equally as PSI at each concentration, possibly because of insufficient membrane permeability of lactacystin.

To examine the mechanism by which proteasome inhibition suppresses basal and TNF-α-induced ET-1 release from cultured ECs, ET-1 mRNA levels were quantitated by Northern blot analysis. TNF-α increased steady-state levels of ET-1 mRNA under basal condition, in a time-dependent manner. Levels of ET-1 mRNA were maximal over 2–4 h. These results are similar to those of Marsden and Brenner (24). When measured at 4 h, 1 μM PSI suppressed the basal and TNF-α-induced ET-1 mRNA expression, to a degree similar to those seen in ET-1 release from ECs, respectively. Therefore, it is most likely that the suppression of ET-1 release by PSI is accompanying an attenuation of ET-1 mRNA expression, under basal and TNF-α-stimulated conditions.

It has been found that TNF-α stimulates ET-1 mRNA expression without depending upon new protein synthesis (24); however, little is known of the transcriptional regulation of ET-1 gene by TNF-α. On the other hand, it is known that treatment of ECs with a variety of stimuli including TNF-α results in the rapid activation of NF-κB (15), which is found in the cytoplasm of most cells as an inactive complex bound to an inhibitory protein, IκB, through the phosphorylation of IκB, and its subsequent proteolytic degradation by the proteasome-dependent proteolytic pathway (6, 7). Since it remains to be determined if TNF-α-stimulated NF-κB activation regulates ET-1 gene transcription, we examined the induction of NF-κB activation by TNF-α and whether this induction could be suppressed by proteasome inhibitors. Our results showed that TNF-α-induced NF-κB activation occurred rapidly, as it was detectable 5 min after treatment, and that both PSI and lactacystin efficiently suppressed the TNF-α-induced NF-κB activation. Thus, the mechanism by which proteasome inhibition attenuates the ET-1 release and its mRNA expression induced by TNF-α is probably attributed to the suppression of enhanced ET-1 transcription via the activation of NF-κB.

In the present study, we found that basal ET-1 release and its mRNA expression in ECs were not completely but partially decreased by proteasome inhibition. In addition, we could detect the suppression of NF-κB activation by two proteasome inhibitors used, when gels for EMSA were exposed for longer periods. Thus, it seems that proteasome is only partly involved in the basal transcription of ET-1 gene in ECs, possibly via NF-κB activation, although further quantitative experiments are required to clarify how much NF-κB activation can be responsible for the steady-state transcription of ET-1 gene in ECs.

In the activation process of NF-κB, reactive oxygen species (ROS) is the common signal for various stimuli such as TNF-α, interleukin-1 and phorbol myristate acetate (25). TNF-α has been demonstrated to increase ROS production in mesangial cells (26), as well as augmenting ET-1 release and its gene expression in these cells (27) and in ECs (24). There is also a report showing that incubation with xanthine/xanthine oxidase or H2O2 augments ET-1 mRNA levels in human mesangial cells (28). From these findings, it is likely that a link exists between oxidative stress-dependent NF-κB activation and ET-1 production. Among many antioxidants, PDTC is known to inhibit the activation of NF-κB without affecting the induction of another transcriptional factor activator protein-1 (16). α-LA has been demonstrated to reduce TNF-α-induced NF-κB activation (17). It has furthermore been reported that PDTC prevents ROS from activating IκB kinases (7), whereas α-LA inhibits a step behind phosphorylation of IκB (29). Although the inhibitory mechanism of these antioxidants on NF-κB activation differs from that of proteasome inhibitors, each antioxidant had a significant inhibitory effect on basal and TNF-α-induced ET-1 release from EC. These findings raise the possibility that inhibition
of any step in NF-κB activation results in the reduction of ET-1 release from ECs. Recently, we have also obtained the results that another NF-κB suppressor BAY 11-7082, which is known to inhibit IκB phosphorylation (30), markedly reduces basal and TNF-α-induced ET-1 production at the transcriptional level (M. Ohkita et al., unpublished observation). Taken together, it is most likely that the inhibition of NF-κB activation causes the suppression of ET-1 production and that NF-κB functions as a crucial factor in ET-1 production in ECs.

Our previous in vivo studies showed that PSI has anti-hypertensive effects on DOCA-salt-induced hypertension (8) and that PSI attenuated the increased aortic ET-1 content in DOCA-salt hypertensive rats (10). A recent study revealed that increased renal NF-κB and aortic ROS production occurs in DOCA-salt hypertension and that PDTC attenuates the NF-κB activation and ROS production as well as systolic blood pressure in this model of hypertension (31). Taken together with the present data, it seems that like proteasome inhibitors and antioxidants, drugs that can inhibit the NF-κB activation may exhibit beneficial effects on various diseases that result from aberrant ET-1 production.

In conclusion, our results indicate that the inhibition of proteasome attenuates the ET-1 production in ECs and that a proteasome-dependent-proteolytic pathway is involved in the enhancement of ET-1 production, possibly through the activation of NF-κB. Thus, proteasome may represent a potential target to be included in studies on ET-1 related diseases.

Acknowledgments

The authors are grateful to Mariko Ohara for critical comments and language assistance.

REFERENCES

1 Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K and Masaki T: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332, 411 – 415 (1988)
2 Miyauchi T and Masaki T: Pathophysiology of endothelin in the cardiovascular system. Annu Rev Physiol 61, 391 – 415 (1999)
3 Tanaka K: Proteasomes: structure and Biology. J Biochem 123, 195 – 204 (1998)
4 Hilt W and Wolf DH: Proteasomes: destruction as a programme. Trends Biochem Sci 21, 96 – 102 (1996)
5 Oikawa T, Sasaki T, Nakamura M, Shimamura M, Tanahashi N, Ōmura S and Tanaka K: The proteasome is involved in angiogenesis. Biochem Biophys Res Commun 246, 243 – 248 (1998)
6 Palombella VJ, Rando OJ, Goldberg AL and Maniatis T: The ubiquitin-proteasome pathway is required for processing the NF-κB precursor protein and the activation of NF-κB. Cell 78, 773 – 785 (1994)
7 Traenckner EB-M, Wilk S and Baeuerle PA: 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 (1994)
8 Takaoka M, Okamoto H, Ito M, Nishioka M, Kita S and Matsumura Y: Anti-hypertensive effect of a proteasome inhibitor in DOCA-salt hypertensive rats. Life Sci 63, PL65 – PL70 (1998)
9 Schiffrin EL: Role of endothelin-1 in hypertension. Hypertension 34, 876 – 881 (1999)
10 Okamoto H, Takaoka M, Ohkita M, Itoh M, Nishioka M and Matsumura Y: A proteasome inhibitor lessens the increased aortic endothelin-1 content in deoxycorticosterone acetate-salt hypertensive rats. Eur J Pharmacol 350, R11 – R12 (1998)
11 Matsumura Y, Ikehara R, Takaoka M and Morimoto S: Conversion of porcine big endothelin to endothelin by an extract from the porcine aortic endothelial cells. Biochem Biophys Res Commun 167, 203 – 210 (1990)
12 Hexum TD, Hoefer C, Rivier JE, Baird A and Brown MR: Characterization of endothelin secretion by vascular endothelial cells. Biochem Biophys Res Comm 167, 294 – 300 (1990)
13 Mitsumori N, Akashi C, Odagiri J and Matsumura Y: Effects of endogenous and exogenous nitric oxide on endothelin-1 production in cultured vascular endothelial cells. Eur J Pharma col 364, 65 – 73 (1999)
14 Schreiber E, Matthias P, Müller MM and Schaffner W: Rapid detection of octamer binding proteins with ‘mini-extracts’, prepared from a small number of cells. Nucleic Acids Res 17, 6419 (1989)
15 Collins T, Read MA, Neish AS, Whiteley MZ, Thanos D and Maniatis T: Transcriptional regulation of endothelial cell adhesion molecules: NF-κB and cytokine-inducible enhancers. FASEB J 9, 899 – 909 (1995)
16 Schreck R, Meier B, Männel DN, Drege W and Baeuerle PA: Dithiocarbamates as potent inhibitors of nuclear factor κB activation in intact cells. J Exp Med 175, 1181 – 1194 (1992)
17 Packer L: α-Lipoic acid: a metabolic antioxidant which regulates NF-κB signal transduction and protects against oxidative injury. Drug Metab Rev 30, 245 – 275 (1998)
18 Figueiredo-Pereira ME, Berg KA and Wilk S: A new inhibitor of the chymotrypsin-like activity of the multicatalytic proteinase complex (20S proteasome) induces accumulation of ubiquitin-protein conjugates in a neuronal cell. J Neurochem 63, 1578 – 1581 (1994)
19 Girschavge JM, Wilk S and Ignarro LJ: Inhibitors of proteasome pathway interfere with induction of nitric oxide synthase in macrophage by blocking activation of transcription factor NF-κB. Proc Natl Acad Sci USA 93, 3308 – 3312 (1996)
20 Takaoka M, Itoh M, Hayashi S, Kuro T and Matsumura Y: Proteasome participates in the pathogenesis of ischemic acute renal failure in rats. Eur J Pharmacol 384, 43 – 46 (1999)
21 Tsujinaka T, Kajiwara Y, Kambayashi J, Sakon M, Higuchi N, Tanaka T and Mori T: Synthesis of a new cell penetrating calpain inhibitor (calpeptin). Biochem Biophys Res Commun 153, 1201 – 1208 (1988)
22 Fenteany G, Standaert RF, Lane WS, Choi S, Corey EJ and Schreiber SL: Inhibition of proteasome activities and subunit-specific amino-terminal threonine modification by lactacystin. Science 268, 726 – 731 (1995)
23 Dick LR, Cruikshank AA, Grenier L, Melandri FD, Nunes SL and Stein RL: Mechanistic studies on the inactivation of the proteasome by lactacystin. J Biol Chem 271, 7273 – 7276 (1996)
24 Marsden PA and Brenner BM: Transcriptional regulation of endothelin-1 gene by TNF-α. Am J Physiol 262, C854 – C861 (1992)

25 Sen CK and Packer L: Antioxidant and redox regulation of gene transcription. FASEB J 10, 709 – 720 (1996)

26 Radeke HH, Meier B, Topley N, Flöge J, Habermehl GG and Resch L: Interleukin 1-α and tumor necrosis factor-α induce oxygen radical production in mesangial cells. Kidney Int 37, 767 – 775 (1990)

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

28 Hughes AK, Stricklett PK, Padilla E and Kohan DE: Effect of reactive oxygen species on endothelin-1 production by human mesangial cells. Kidney Int 49, 181 – 189 (1996)

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

30 Pierce JW, Schoenleber R, Jesmok G, Best J, Moore SA, Collins T and Gerritsen ME: Nobel inhibitors of cytokine-induced IkB phosphorylation and endothelial cell adhesion molecule expression show anti-inflammatory effects in vivo. J Biol Chem 272, 21096 – 21103 (1997)

31 Beswick RA, Zhang H, Marable D, Catravas JD, Hill WD and Webb RC: Long-term antioxidant administration attenuates mineralocorticoid hypertension and renal inflammatory response. Hypertension 37, 781 – 786 (2001)