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Lipopolysaccharide induces endothelial cell apoptosis via activation of Na^+/H^+ exchanger 1 and calpain-dependent degradation of Bcl-2

Yuxi Zhao, Guimei Cui, Nana Zhang, Zengshan liu, Wanchun Sun, Qisheng Peng *

Key Laboratory for Zoonosis Research, Ministry of Education, Institute of Zoonosis, Jilin University, Changchun 130062, People's Republic of China

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ABSTRACT

The calcium-dependent protease calpain is involved in lipopolysaccharide (LPS)-induced endothelial injury. The activation of Na^+/H^+ exchanger (NHE) is responsible to increase intracellular Ca^{2+} (Ca_i^{2+}) in cardiovascular diseases. Here we hypothesized that activation of NHE mediates LPS-induced endothelial cell apoptosis via calcium-dependent calpain pathway. Our results revealed that LPS-induced increases in NHE activity are dependent on NHE1 in human umbilical vein endothelial cells (HUVECs). Treatment of HUVECs with LPS increased the NHE1 activity in a time-dependent manner associated with the increased Ca_i^{2+}, which resulted in enhanced calpain activity as well as HUVECs apoptosis via NHE1-dependent degradation of Bcl-2.

1. Introduction

Lipopolysaccharide (LPS) is a component of the outer membrane Gram-negative bacteria and elicits inflammatory responses in immune and non-immune cells including endothelial cells [1]. The endothelial cell is a prime target of the LPS molecule, and vascular complications of septic shock due to Gram-negative bacteria are related to endothelial injury [2,3]. Indeed, LPS-induced systemic organ failure is triggered initially by vascular endothelial injury, characterized by vascular occlusion, perivascular accumulation of leukocytes and cell death [4]. Recently, it has been demonstrated that LPS induces endothelial cell injury by activating calpain [5]. However, the molecular mechanism of LPS-induced activation of the death pathway in endothelial cells is not completely understood.

Na^+/H^+ exchanger 1 (NHE1), a ubiquitously expressed protein on mammalian plasma membranes [6], is known to involve cell apoptosis [7,8]. NHE1 exchanges intracellular H^+ for extracellular Na^+ to regulate intracellular pH (pH_i) value and the concentration of intracellular Na^+ (Na_i^+) [9]. The activation of NHE1 increases Na_i^+ concentration that leads to Ca^{2+} overload through the Na^+/Ca^{2+} exchanger, which is assumed to be the crucial factor in cell injury [10]. The increased Ca_i^{2+} concentration automatically activates calpain, a calcium-dependent protease [11]. Many proteins in cells are the substrates of calpain, including anti-apoptotic family member, Bcl-2 [12]. Previous studies have shown that inhibition of NHE1 has anti-apoptotic effects and LPS-induced apoptosis is dependent on changes in Bcl-2 [7,8,13]. These findings support the hypothesis that LPS-induced apoptosis via Ca^{2+}/calpain-dependent Bcl-2 degradation is mediated by NHE1.

In this study, we demonstrate that LPS stimulates a calpain-mediated apoptosis pathway in HUVECs, and the LPS-induced apoptosis in endothelial cells is mediated through activation of NHE1. The study provides new mechanisms underlying LPS-induced apoptosis in endothelial cells.

2. Materials and methods

2.1. Materials

Zllal (benzyloxycarbonyl-leucyl-leucinal) was purchased from Biomol Research Laboratories (Plymouth Meeting, USA). Calcium chelator, BAPTA, was purchased from Invitrogen. 2-carboxyethyl-5(6)-carboxyfluorescein (BCECF) was purchased from Calbiochem (Billerica, USA). Pan-Caspase Inhibitor, Z-VAD-FMK, was purchased from KAMIYA BIOMEDICAL (Seattle, USA). Cariporide and other chemicals were purchased from Sigma (Shanghai, China). Anti-Bcl-2, β-actin, caspase-3 and 2nd antibodies were purchased from Cell Signaling. Anti-NHE1 antibody was purchased from Santa Cruz.

2.2. Culture of human umbilical vein endothelial cells (HUVECs)

HUVECs were purchased from ATCC and cultured according to the method described previously [14]. HUVECs were grown in EBM (Clonetics Inc. Walkersville, MD) supplemented with 12.5 mg/mL ECGF, 10% FBS, 100 U/mL penicillin, 100 μg/mL

* Corresponding author. Fax: +86 431 87836720.
E-mail address: Peng_Qi_sheng@yahoo.com (Q. Peng).

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streptomycin, and 1 mg/mL hydrocortisone. Cells were subcultured when 80–90% confluent. Cells at passages 3–8 were used for all experiments.

2.3. Experimental design

Eighty to ninety percent of confluent HUVECs were serum starved overnight prior to stimulation with LPS (100 ng/ml) for 1, 4, 8, 16, 24, and 48 h. For the dose-course study of cariporide, LPS-treated HUVECs were co-incubated with cariporide for 24 h in different doses (1, 5, 10, 20 μM). For studying the intervention of other drugs, HUVECs were treated with cariporide (10 μM), Zlla1 (50 μM), Z-VAD-FMK (20 μM), or BAPTA (0.5 mM) in presence or absence of LPS (100 ng/ml) for 24 h. After treatment, cells were subjected to detect NHE activity, Ca_i^{2+} concentration, calpain activity, the Bcl-2 protein level, Bcl-2 mRNA level, and cell apoptosis by NH_4Cl pulse method, Fluo-4 fluoresence, fluorogenic peptide substrate, Western blot, RT-PCR, and TUNEL, respectively.

2.4. RNA interference

NHE1-RNAi experiments were performed according to the method described by Mo et al. [15]. Briefly, the NHE1-targeted double-stranded small interfering RNAs (NHE1-A, CGAAGAGAUCCACACACAGTT; NHE1-B, CUGUGUGUGGAUCUCUUCGTT) were chemically synthesized by Invitrogen. When the HUVECs reached 80–90% confluence, transfection of the NHE1 SiRNA duplex (final concentration, 40 nM) was carried out using Lipofectamine 2000 (Invitrogen). The negative control was set up using the 21-nucleotide RNA oligonucleotide that corresponded to the coding sequence of luciferase. Suppression of expression of endogenous NHE1 by the SiRNAs was determined by Western blot. After 24 h transfection, HUVECs were used for further experiments.

2.5. Western blot

HUVECs were lysed in 1× cell-lysis buffer (Cell Signaling). The protein content was assayed by BCA protein assay reagent (Pierce, USA). Twenty micrograms of protein was loaded to SDS–PAGE and then transferred to membrane. Membrane was incubated with a 1:1000 dilution of primary antibody, followed by a 1:2000 dilution of horseradish peroxidase-conjugated secondary antibody. Protein bands were visualized by ECL (GE Healthcare). The band densities were measured by densitometry (model GS-700, Imaging Densitometer; Bio-Rad). The background was subtracted from the calculated area.

2.6. RT-PCR analysis for Bcl-2 mRNA

Total RNA was extracted from cells by TRIZOL (Life Technologies, USA) reagent according to the manufacturer's protocol. RT-PCR analysis for Bcl-2 mRNA was performed following previous described procedure [16]. Primer sequences were: Bcl-2 sense, 5′-GTGGATGACTGAGTACCTGAACC-3′; Bcl-2 antisense, 5′-AGCCAGG AGAAATCAAACAGAG-3′; GAPDH sense, 5′-TCATTTCCTGGTATGACAACG-3′; GAPDH antisense, 5′-TTACTCCTTGG AGGCCATGT-3′. The PCR protocol for Bcl-2 consisted of: (1) denaturation at 94 °C for 5 min, (2) 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 2 min, (3) final extension at 72 °C for 15 min. The protocol for GAPDH was the same as Bcl-2 except for 1 min annealing at 65 °C and amplification for 25 cycles.

2.7. Measurement of NHE activity in HUVECs

NHE activity in HUVECs was assayed with NH_4Cl pulse method [17]. Briefly, HUVECs were washed with HCO_3^--free HBS buffer (mM: NaCl, 140; KCl, 5; $CaCl_2$, 1; $MgCl_2$, 1; glucose, 5; HEPES, 6. pH 7.4). After incubation of HUVECs with HBS containing 10 μM BCECF at 37 °C for 30 min and removal of free-BCECF by washing with HBS, 40 mM NH_4Cl was added into HBS, incubated for 5 min, and washed out with Na^+-free HBS buffer. Cells were acid-loaded and pH_i decreased. When 100 mM NaCl was added, intracellular H^+ was pumped out via Na^+/H^+ exchange and pH_i increased linearly during the initial 40 s. This initial rate of pH_i recovery (dpH_i/dt) was considered to reflect the Na^+/H^+ exchange activity.

2.8. Calpain activity assay

The calpain activity was measured by using the fluorogenic peptide Suc-Leu-Leu-Val-Tyr-AMC as a substrate following the procedure described previously with slight modification [18]. Briefly, cells were cultured in 24-well plates in medium with different treatments. After being washed twice with PBS, fluorogenic peptide was added to a final concentration of 80 μM in PBS. Immediately, fluorescence was recorded at 2 min intervals for 20 min at excitation 360 nm and emission 460 nm by a Synergy HT Multi-Detection Microplate Reader (BIO-TEK Instruments Inc.). The initial rate of peptidyl-AMC hydrolysis was used as the velocity of enzyme activity.

2.9. Measurement of Ca_i^{2+} concentration

The Ca_i^{2+} concentration was measured by using a Fluo-4 NW kit (Invitrogen) according to previous described procedure [19]. Briefly, the cell culture medium was aspirated after treatment, washed with HEPES buffer (pH 7.4) once, and 1 ml of HEPES buffer containing fluorescent dye was added to cultured cells. After 30 min incubation, fluorescence strength was measured in wavelength of excitation/emission of 485/520 nm.

2.10. Apoptosis assay by TUNEL in HUVECs

HUVECs were fixed with 4% paraformaldehyde in PBS. Cell apoptosis was assessed by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling staining (TMR red) by using a kit (Roche Applied Science) and following the procedure described previously [20]. The percentage of apoptosis was calculated from the number of terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling-positive cells divided by the total number of cells counted.

2.11. Statistical analysis

Statistical analysis was performed using SPSS10.0 software. Data are expressed as mean ± SEM. The statistical significance of differences was evaluated by using one-way ANOVA followed by the Student's *t*-test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. LPS-induced increases in NHE activity are dependent on NHE1 in HUVECs

Early studies have shown that LPS induces endothelial cell apoptosis and endothelial dysfunction, which contribute to cardiovascular disease [21]. The NHE plays an important role in

myocardial damage during ischemia and reperfusion and has recently been implicated as a mediator of cardiac hypertrophy [6]. We first determined whether LPS activates NHE in HUVECs. As shown in Fig. 1A, LPS increased NHE activity beginning at 4 h. The increased NHE activity induced by LPS reached the peak at 16 to 24 h, and began to go down from 48 h incubation. Considering that the NHE1 isoform exists on the plasma membrane of all mammalian cells and is the predominant isoform in HUVECs [15,22], we hypothesized that NHE activity depends on NHE1. To prove our hypothesis, LPS-treated HUVECs were co-incubated with cariporide (a specific NHE1 inhibitor) for 24 h in different doses. Our data indicated that cariporide inhibited the LPS-induced increase of NHE activity in a dose-dependent manner and 10 μM cariporide could abolish NHE activity (Fig. 1B). In order to rule out the potential off-target effects of cariporide, NHE1 knockdown was performed to further investigate whether LPS-induced increases in NHE activity is dependent on NHE1. As shown in Fig. 1C, NHE1 activity with NHE1 SiRNA was far lower than that with

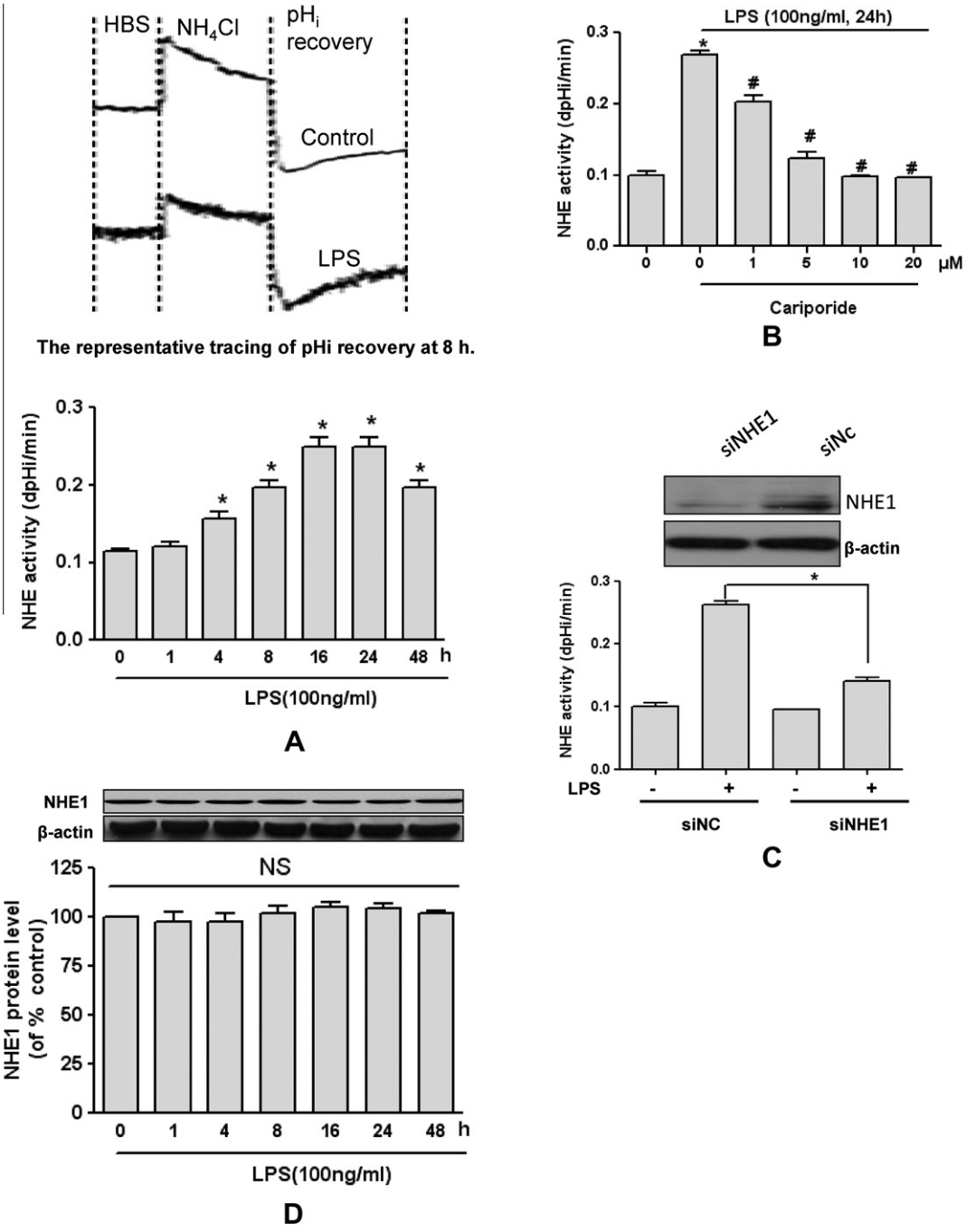

Fig. 1. LPS-induced increases in NHE activity are dependent on NHE1 in HUVECs. HUVECs were incubated with LPS (100 ng/ml) for 1, 4, 8, 16, 24, and 48 h. The representative tracing of pHi recovery was shown (control and LPS group at 8 h) (A, top), and NHE activity was measured (A, bottom). (B) HUVECs were incubated with LPS (100 ng/ml) in presence or absence of cariporide (1, 5, 10, 20 μM) for 24 h, and NHE activity was measured. (C) HUVECs were transfected with NHE1 SiRNA (SiNHE1) or NHE1 negative control oligonucleotide (SiNC) for 24 h. HUVECs were incubated with or without LPS (100 ng/ml) for another 24 h, and NHE activity was measured. (D) NHE1 protein expression. Data are expressed as mean ± SEM ($n = 5$). The blot is a representative from 3 independent experiments. $^{*}P < 0.05$ vs Control, $^{\#}P < 0.05$ vs LPS alone. NS is indicated as no significant difference.

control SiRNA. Interestingly, LPS did not alter NHE1 protein expression throughout the treatment period (Fig. 1D). These data suggest that LPS-induced increases in NHE activity depend on NHE1 in HUVECs.

3.2. LPS increases intracellular calcium level via activation of NHE1

The activation of NHE1 increases Na_i^+ that leads to Ca^{2+} overload through the Na^+/Ca^{2+} exchanger, which is assumed to be the crucial factor in many diseases, such as ischemia [23] and atherosclerosis [24]. Here we further investigated whether activation of NHE1 by LPS results in the increased intracellular calcium level. As shown in Fig. 2A, LPS time-dependently increased intracellular calcium levels in HUVECs. In addition, the LPS-induced increase of intracellular calcium level was reversed by cariporide treatment in a dose-dependent manner (Fig. 2B). These data reveal that LPS-enhanced intracellular calcium level is NHE1-dependent.

3.3. LPS-enhanced calpain activity is both NHE1 and calcium-dependent

Calpains are a family of Ca^{2+}-dependent cysteine proteases found in mammals and many lower organisms [19,25]. In the presence of elevated Ca_i^{2+} concentrations, calpain is activated and regulates wide range of cellular functions by degrading a variety of proteins, such as HSP90 and Bcl-2 [12,26,27]. We next determined whether LPS also induced calpain activity in HUVECs. As shown in Fig. 2C, LPS time-dependently increased calpain activity in HUVECs.

In Fig. 2D and E, inhibition of NHE1 by cariporide or chelation of intracellular Ca^{2+} by BAPTA effectively abolished the LPS-increased

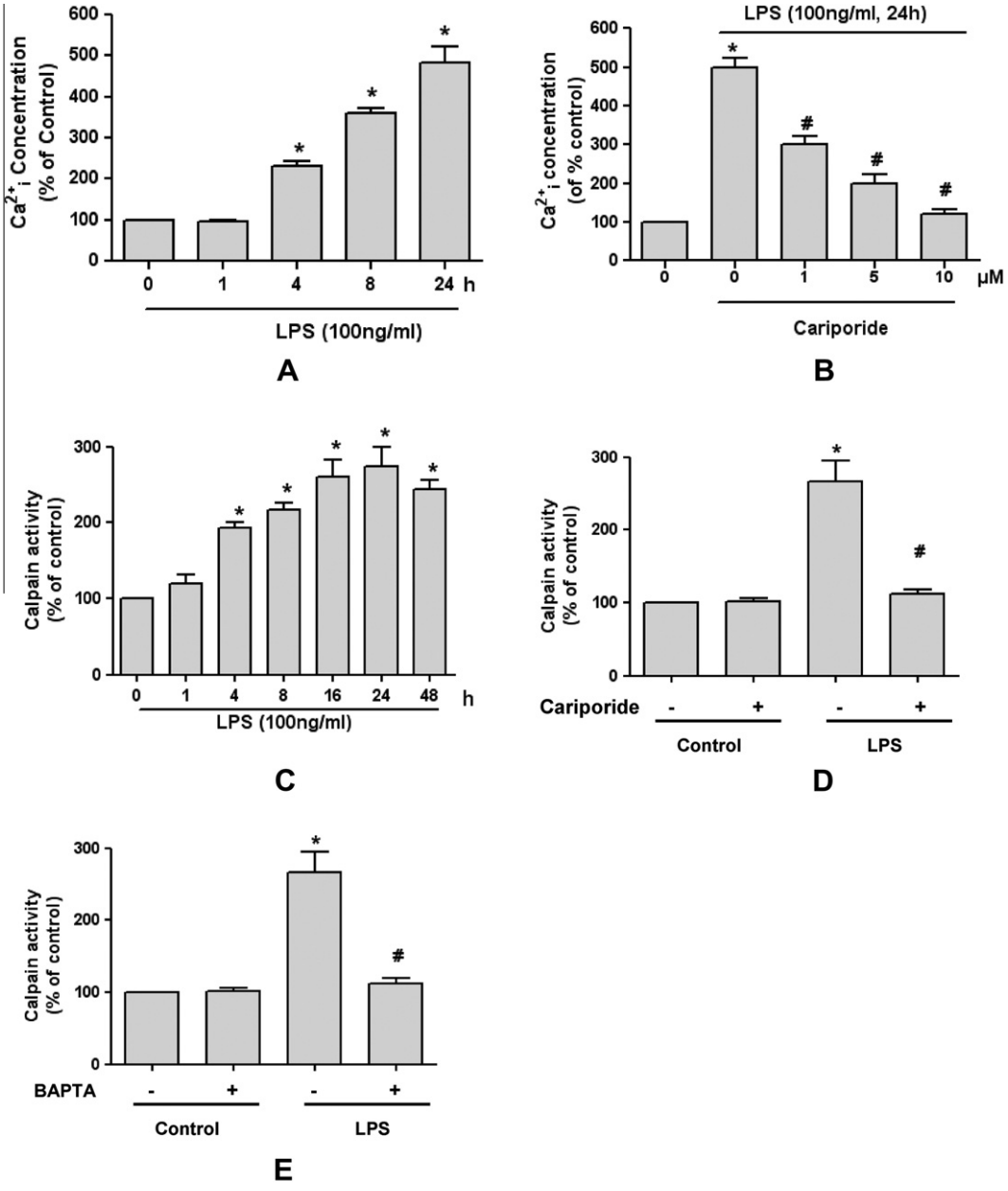

Fig. 2. LPS increases intracellular calcium levels and calpain activity via activation of NHE1. (A) HUVECs were incubated with LPS (100 ng/ml) for 1, 4, 8, and 24 h. (B) HUVECs were incubated with LPS (100 ng/ml) in presence or absence of cariporide (1, 5, 10 μM) for 24 h. The intracellular calcium concentration of A and B was determined by Fluo-4 fluorescence. (C) HUVECs were incubated with LPS (100 ng/ml) for 1, 4, 8, 16, 24, and 48 h (D) HUVECs were incubated with LPS (100 ng/mL) for 24 h in the absence or presence of cariporide (10 μM). (E) HUVECs were incubated with LPS (100 ng/mL) for 24 h in the absence or presence of BAPTA (0.5 mM). Calpain activity (C–E) was assayed by using the fluorogenic peptide substrate. Control group was defined as 100%. Data are expressed as mean ± SEM ($n = 5$). $^*P < 0.05$ vs control, $^\#P < 0.05$ vs LPS alone.

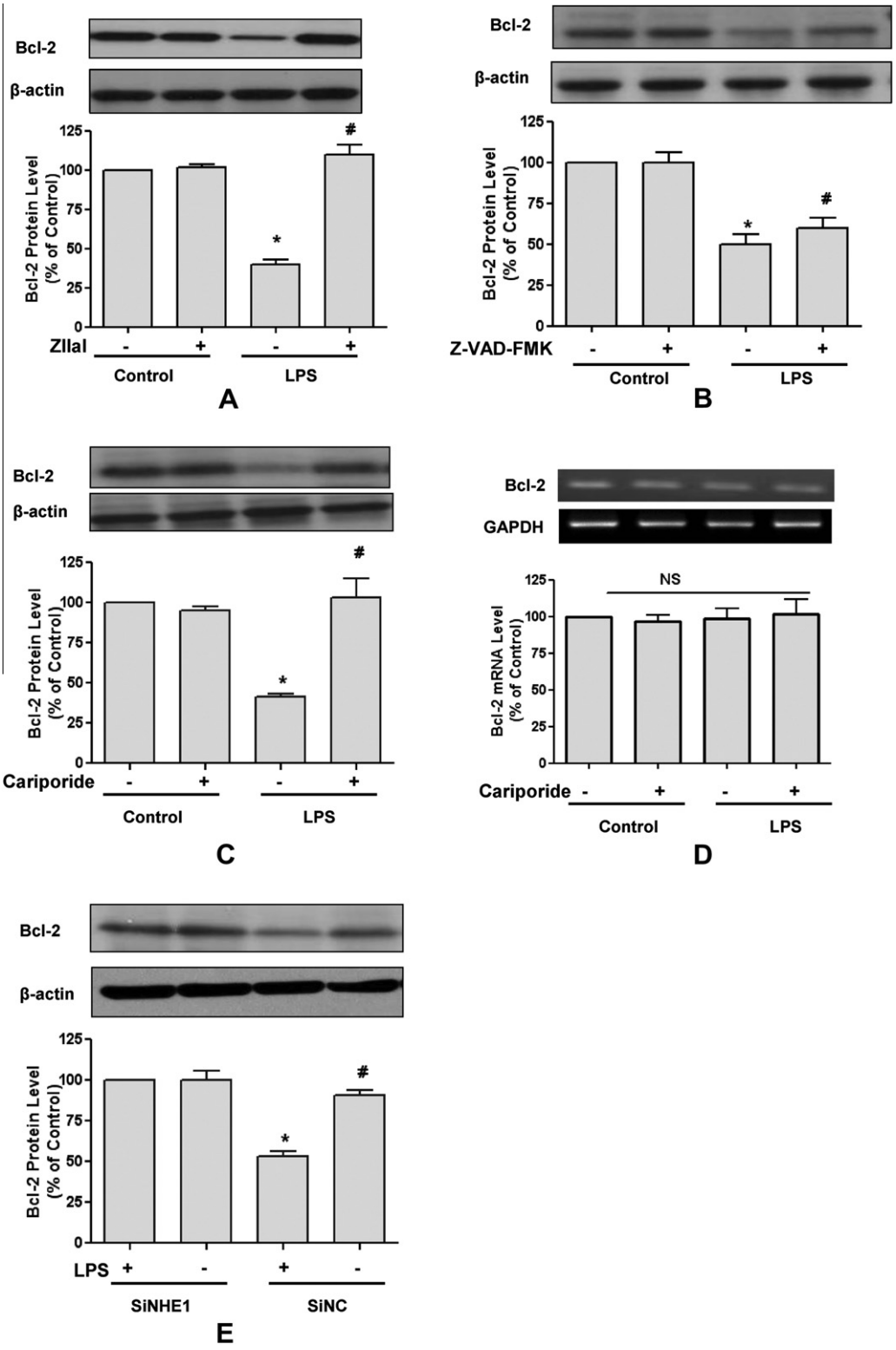

Fig. 3. LPS induces NHE1 activation-dependent Bcl-2 degradation. HUVECs were incubated with LPS (100 ng/mL) for 24 h in the absence or presence of ZIlal (50 μM) (A) or Z-VAD-FMK (20 μM) (B). Cells were subjected to detect Bcl-2 protein level by Western blot. (C, D) HUVECs were incubated with LPS (100 ng/mL) for 24 h in the absence or presence of cariporide (10 μM) Cells were subjected to detect Bcl-2 protein level by Western blot and Bcl-2 mRNA level by RT-PCR. (E) HUVECs were transfected with SiNHE1 or SiNC for 24 h. HUVECs were incubated with or without LPS (100 ng/mL) for another 24 h. Cells were subjected to detect Bcl-2 protein level by Western blot. Data are expressed as mean ± SEM. The blot is a representative from 3 independent experiments. $^{*}P < 0.05$ vs control, $^{\#}P < 0.05$ vs LPS alone, NS is indicated as no significant difference.

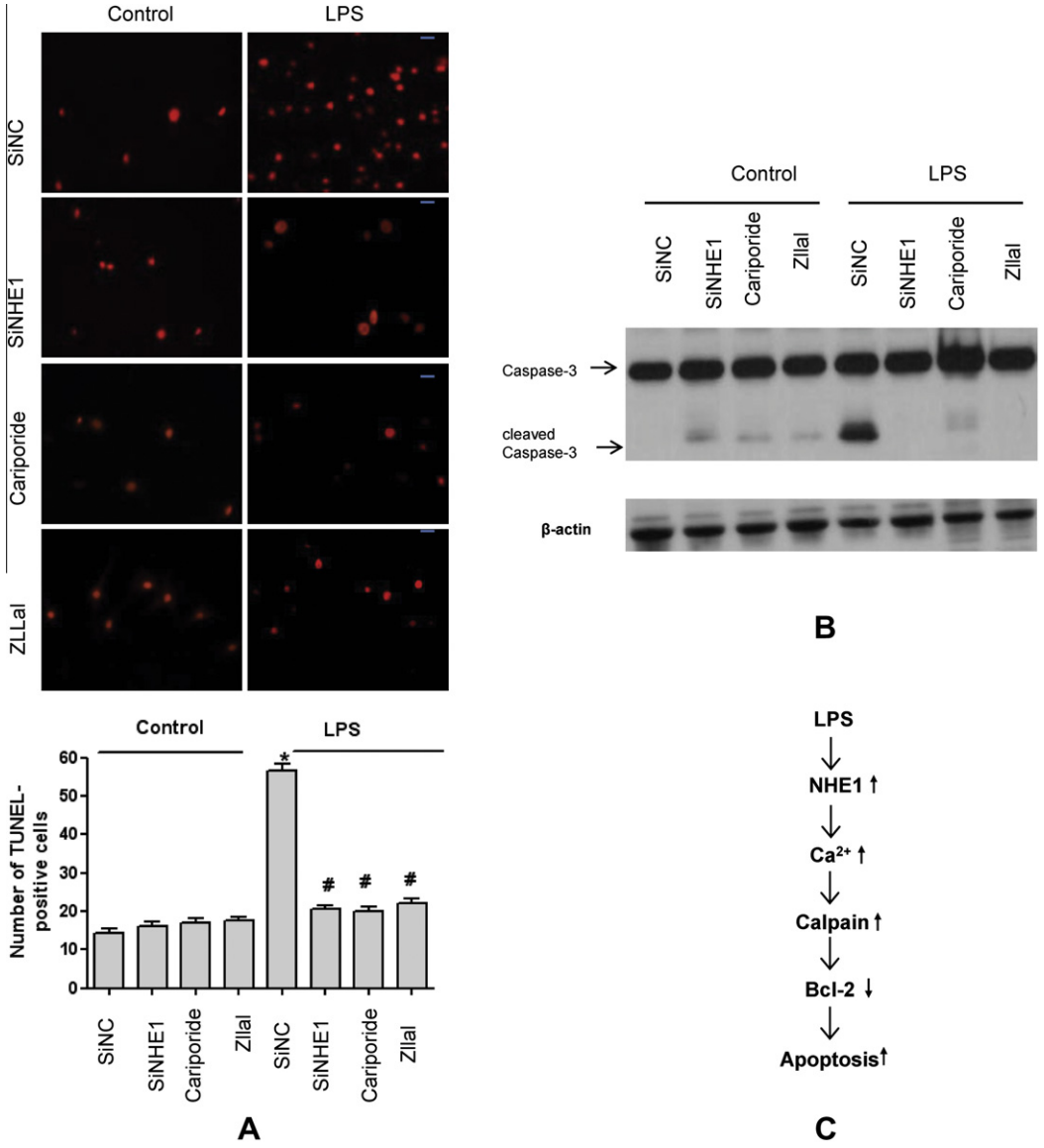

Fig. 4. NHE1 activation is required for LPS-induced HUVECs apoptosis. HUVECs were incubated with cariporide (10 μM) or Zllal (50 μM) for 24 h in presence or absence of LPS (100 ng/mL), or HUVECs transfected with SiNHE1 or SiNC were incubated with or without LPS (100 ng/mL) for 24 h. The cells apoptosis was assayed by TUNEL method (A) and cleaved Caspase-3 assay by Western blot (B). The number of TUNEL positive cells was from total 100 cells (scale bar = 20 μm). Data are expressed as mean ± SEM ($n = 5$). $^{*}P < 0.05$ vs control, $^{\#}P < 0.05$ vs LPS alone. The blot is a representative from 3 independent experiments. (C) Proposed scheme of LPS-induced endothelial cell apoptosis via the NHE1/calcium/calpain/Bcl-2 pathway.

calpain activity. Taken together, these data indicate that LPS induces activation of NHE1, resulting in elevations of Ca_i^{2+}, and causing activation of calpains.

3.4. LPS induces NHE1 activation-dependent Bcl-2 degradation

Considering that calpain activation promotes decrease of Bcl-2 proteins thereby triggering the intrinsic apoptotic pathway [12], HUVECs were treated with LPS in the absence or presence of calpain specific inhibitor, Zllal, to identify whether NHE1-induced calpain activation mediates Bcl-2 degradation. As shown in Fig. 3A, inhibition of calpain by Zllal abolished the LPS-decreased Bcl-2 protein levels, in contrast to Zllal treated HUVECs, LPS induced significant degradation of Bcl-2 in naïve cells, suggesting that LPS-decreased Bcl-2 protein is calpain-dependent.

It has been proved that caspase activation play an essential role in cells apoptosis [28]. We next tested whether decrease in Bcl-2 protein is a secondary effect of caspase activation and not a direct effect of calpain on Bcl-2. Our data indicated that Z-VAD-FMK, Pan-Caspase Inhibitor, did not block the LPS-induced reduction of Bcl-2 protein (Fig. 3B). Thus, Bcl-2 is degraded in a caspase-independent manner in LPS treated HUVECs.

Because LPS-induced NHE1 activation results in enhanced calpain activity, we then determined whether degradation of Bcl-2 depends on NHE1 activation. As shown in Fig. 3C, treatment of HUVECs with LPS reduced the protein levels of Bcl-2. Inhibition of NHE1 by cariporide abolished the LPS-decreased Bcl-2 protein levels.

Reductions in BCL-2 protein could be due to LPS-induced repression of Bcl-2 gene transcription. In order to exclude this possibility, we checked the mRNA of Bcl-2 during HUVEC treatment. As indicated in Fig. 3D, LPS alone did not change Bcl-2 mRNA level. Cariporide did not alter the mRNA level of Bcl-2 in basal condition or LPS-treated HUVECs, either. These data suggested that LPS decreased Bcl-2 protein levels, likely via protein degradation, and not by decreasing the mRNA biosynthesis.

To further confirm that LPS-induced Bcl-2 degradation is dependent on NHE1, HUVECs were transfected with SiRNA NHE1 to test

the effect of Bcl-2 expression changes in response to LPS stimulation. Bcl-2 degradation observed with knockdown of NHE1 in HUVECs agrees with the data that inhibition of NHE1 activation abolishes LPS-induced down-regulation of Bcl-2 protein. Consistently, Bcl-2 protein was significantly degraded in HUVECs transfected with negative control of SiRNA NHE1 (Fig. 3E). Taken collectively, NHE1 activation is required for LPS-induced Bcl-2 degradation in HUVECs.

3.5. NHE1 activation is required for LPS-induced HUVECs apoptosis

Given the LPS-induced degradation of anti-apoptotic protein Bcl-2, we analyzed apoptosis rates in LPS-treated HUVECs. As shown in Fig. 4A, LPS increased the apoptotic rate of HUVECs transfected with SiNC detected by TUNEL staining. Knockdown of Bcl-2 by SiNHE1 significantly inhibited LPS-induced apoptosis of HUVECs while having no effects on LPS untreated cells. Cariporide alone did not affect the apoptosis in basal condition, but inhibited LPS-induced apoptosis in HUVECs. Consistent with the effect of cariporide, Zllal obviously inhibited LPS-induced apoptosis of HUVECs. Cleavage of caspase-3 assay also verified that LPS-induced HUVEC apoptosis is dependent on NHE1 activation (Fig. 4B).

4. Discussion

The current study demonstrates that LPS induces vascular endothelial cell apoptosis by triggering calpain-dependent degradation of Bcl-2 via activating NHE1. Inhibition of NHE1 reverses LPS-induced increase of calcium concentration and calpain activity. In addition, inhibition of NHE1 by cariporide or RNA interference blocked the decrease of Bcl-2 degradation caused by LPS. These results strongly suggest that NHE1 is required for LPS-induced endothelial cell apoptosis via calcium-dependent protease calpain (Fig. 4C).

The calpains are a family of calcium-dependent protease that acts independently of the proteasome pathway and cleave a number of cellular substrates, including kinases, phosphatases, transcription factors, and cytoskeletal proteins [29]. In this study, we found that LPS increased intracellular calcium as well as calpain activity in vascular endothelial cells. In addition, chelation of intracellular free-calcium by BAPTA inhibited the activation of calpain. So we speculated that LPS-induced calpain activation is calcium-dependent because it has been reported that calpain is activated in response to large calcium fluxes [11].

NHE1 is the predominant isoform in endothelial cells, we assume that LPS produced NHE activation dependently of NHE1. A potential mechanism of LPS-induced increase of NHE1 activity is possibly a phosphorylation-dependent increase in the activity of existing exchangers or the activation of dormant membrane-associated exchangers. In fact, Sardet et al. have demonstrated that NHE is rapidly phosphorylated in response to various mitogens and concluded that this phosphorylation of NHE is temporally correlated with its activation [30].

The anti-apoptotic Bcl-2 protein is localized on outer mitochondrial membrane, and functions to prevent cytochrome C release from mitochondria [28,31]. In our results, LPS decreased the level of Bcl-2 protein but not mRNA in HUVECs, however, cariporide or Zllal reversed the effect of LPS. All the results indicate that calpain may play some roles in LPS-induced degradation of Bcl-2, however, which calpain isoform is involved in the degradation of Bcl-2 and whether Bcl-2 is the direct substrate of calpain in LPS-treated HUVECs needs further investigations. In addition, it has been reported that calpain can activate caspase-9, which in turn can cleave and activate effector caspase-3 [32]. However, our data indicated that inhibition of caspase activity still resulted in degradation of Bcl-2, whereas inhibition of calpain completely abolished LPS-decreased Bcl-2 and caspase-3 cleavage, suggesting that Bcl-2 degradation is upstream executioner of HUVECs apoptosis.

In conclusion, NHE1 activation facilitates LPS-induced apoptosis, mediated by calpain-dependent Bcl-2 degradation in HUVECs. Inhibition of NHE1 could be a promising novel approach to halt or even reverse endothelial dysfunction in diabetes or hypertension patients.

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References

[1] C.R. Raetz, Biochemistry of endotoxins, Annu. Rev. Biochem. 59 (1990) 129–170.
[2] M.I. Cybulsky, M.K. Chan, H.Z. Movat, Acute inflammation and microthrombosis induced by endotoxin, interleukin-1, and tumor necrosis factor and their implication in gram-negative infection, Lab. Invest. 58 (1988) 365–378.
[3] J.S. Pober, R.S. Cotran, The role of endothelial cells in inflammation, Transplantation 50 (1990) 537–544.
[4] K.B. Choi, F. Wong, J.M. Harlan, P.M. Chaudhary, L. Hood, A. Karsan, Lipopolysaccharide mediates endothelial apoptosis by a FADD-dependent pathway, J. Biol. Chem. 273 (1998) 20185–20188.
[5] T. Liu, Y. Huang, R.I. Likhotvorik, L. Keshvara, D.G. Hoyt, Protein Never in Mitosis Gene A Interacting-1 (PIN1) regulates degradation of inducible nitric oxide synthase in endothelial cells, Am. J. Physiol. Cell Physiol. 295 (2008) C819–C827.
[6] L. Fliegel, The Na+/H+ exchanger isoform 1, Int. J. Biochem. Cell Biol. 37 (2005) 33–37.
[7] J.R. Schelling, B.G. Abu Jawdeh, Regulation of cell survival by Na+/H+ exchanger-1, Am. J. Physiol. Renal. Physiol. 295 (2008) F625–F632.
[8] C.D. Garciarena, C.I. Caldiz, E.L. Portiansky, G.E. Chiappe de Cingolani, I.L. Ennis, Chronic NHE-1 blockade induces an antiapoptotic effect in the hypertrophied heart, J. Appl. Physiol. (2009).
[9] S. Wang, L. Liu, M. Hu, Y. Liu, Na+/H+ exchanger inhibitor prevented endothelial dysfunction induced by high glucose, J. Cardiovasc. Pharmacol. 45 (2005) 586–590.
[10] D. Wang, K. Dou, Z. Song, Z. Liu, The Na+/H+ exchange inhibitor: a new therapeutic approach for hepatic ischemia injury in rats, Transplant. Proc. 35 (2003) 3134–3135.
[11] D.E. Goll, V.F. Thompson, H. Li, W. Wei, J. Cong, The calpain system, Physiol. Rev. 83 (2003) 731–801.
[12] S. Gil-Parrado, A. Fernandez-Montalvan, I. Assfalg-Machleidt, O. Popp, F. Bestvater, A. Holloschi, T.A. Knoch, E.A. Auerswald, K. Welsh, J.C. Reed, H. Fritz, P. Fuentes-Prior, E. Spiess, G.S. Salvesen, W. Machleidt, Ionomycin-activated calpain triggers apoptosis. A probable role for Bcl-2 family members, J. Biol. Chem. 277 (2002) 27217–27226.
[13] H.L. Wang, I.O. Akinci, C.M. Baker, D. Urich, A. Bellmeyer, M. Jain, N.S. Chandel, G.M. Mutlu, G.R. Budinger, The intrinsic apoptotic pathway is required for lipopolysaccharide-induced lung endothelial cell death, J. Immunol. 179 (2007) 1834–1841.
[14] S. Wang, J. Xu, P. Song, Y. Wu, J. Zhang, H. Chul Choi, M.H. Zou, Acute inhibition of guanosine triphosphate cyclohydrolase 1 uncouples endothelial nitric oxide synthase and elevates blood pressure, Hypertension 52 (2008) 484–490.
[15] X.G. Mo, Q.W. Chen, X.S. Li, M.M. Zheng, D.Z. Ke, W. Deng, G.Q. Li, J. Jiang, Z.Q. Wu, L. Wang, P. Wang, Y. Yang, G.Y. Cao, Suppression of NHE1 by small interfering RNA inhibits HIF-1 alpha-induced angiogenesis in vitro via modulation of calpain activity, Microvasc. Res. 81 (2011) 160–168.
[16] A. Kyronlahti, M. Ramo, M. Tamminen, L. Unkila-Kallio, R. Butzow, A. Leminen, M. Nemer, N. Rahman, I. Huhtaniemi, M. Heikinheimo, M. Anttonen, GATA-4 regulates Bcl-2 expression in ovarian granulosa cell tumors, Endocrinology 149 (2008) 5635–5642.
[17] S.X. Wang, X.Y. Sun, X.H. Zhang, S.X. Chen, Y.H. Liu, L.Y. Liu, Cariporide inhibits high glucose-mediated adhesion of monocyte-endothelial cell and expression of intercellular adhesion molecule-1, Life Sci. 79 (2006) 1399–1404.
[18] Y. Dong, J. Tan, M.Z. Cui, G. Zhao, G. Mao, N. Singh, X. Xu, Calpain inhibitor MDL28170 modulates Abeta formation by inhibiting the formation of intermediate Abeta46 and protecting Abeta from degradation, FASEB J. 20 (2006) 331–333.
[19] S. Wang, Q. Peng, J. Zhang, L. Liu, Na+/H+ exchanger is required for hyperglycemia-induced endothelial dysfunction via calcium-dependent calpain, Cardiovasc. Res. 80 (2008) 255–262.

[20] P. Song, Z. Xie, Y. Wu, J. Xu, Y. Dong, M.H. Zou, Protein kinase Czeta-dependent LKB1 serine 428 phosphorylation increases LKB1 nucleus export and apoptosis in endothelial cells, J. Biol. Chem. 283 (2008) 12446–12455.
[21] D.D. Bannerman, S.E. Goldblum, Mechanisms of bacterial lipopolysaccharide-induced endothelial apoptosis, Am. J. Physiol. Lung Cell. Mol. Physiol. 284 (2003) L899–L914.
[22] M.E. Malo, L. Fliegel, Physiological role and regulation of the Na+/H+ exchanger, Can. J. Physiol. Pharmacol. 84 (2006) 1081–1095.
[23] T. Toda, T. Kadono, M. Hoshiai, Y. Eguchi, S. Nakazawa, H. Nakazawa, N. Higashijima, H. Ishida, Na+/H+ exchanger inhibitor cariporide attenuates the mitochondrial Ca2+ overload and PTP opening, Am. J. Physiol. Heart Circ. Physiol. 293 (2007) H3517–H3523.
[24] G. Koliakos, C. Befani, K. Paletas, M. Kaloyianni, Effect of endothelin on sodium/hydrogen exchanger activity of human monocytes and atherosclerosis-related functions, Ann. N.Y. Acad. Sci. 1095 (2007) 274–291.
[25] M. Azuma, T.R. Shearer, The role of calcium-activated protease calpain in experimental retinal pathology, Surv. Ophthalmol. 53 (2008) 150–163.
[26] T.J. Stalker, Y. Gong, R. Scalia, The calcium-dependent protease calpain causes endothelial dysfunction in type 2 diabetes, Diabetes 54 (2005) 1132–1140.
[27] T.J. Stalker, C.B. Skvarka, R. Scalia, A novel role for calpains in the endothelial dysfunction of hyperglycemia, FASEB J. 17 (2003) 1511–1513.
[28] D.R. Green, J.C. Reed, Mitochondria and apoptosis, Science 281 (1998) 1309–1312.
[29] H. Sorimachi, S. Ishiura, K. Suzuki, Structure and physiological function of calpains, Biochem. J. 328 (Pt 3) (1997) 721–732.
[30] C. Sardet, L. Counillon, A. Franchi, J. Pouyssegur, Growth factors induce phosphorylation of the Na+/H+ antiporter, glycoprotein of 110 kD, Science 247 (1990) 723–726.
[31] Y. Terui, Y. Furukawa, J. Kikuchi, S. Iwase, K. Hatake, Y. Miura, Bcl-x is a regulatory factor of apoptosis and differentiation in megakaryocytic lineage cells, Exp. Hematol. 26 (1998) 236–244.
[32] E. Szegezdi, U. Fitzgerald, A. Samali, Caspase-12 and ER-stress-mediated apoptosis: the story so far, Ann. N.Y. Acad. Sci. 1010 (2003) 186–194.