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Effects of Propofol on Cyclic Strain-induced Endothelin-1 Expression in Human Umbilical Vein Endothelial Cells

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Background: **Propofol is one of the most popular intravenous induction agents of general anesthesia. Experimental results revealed that propofol exerted hypotensive and antioxidative effects. However, the intracellular mechanism of propofol remains to be delineated. The aims of this study were to test the hypothesis that propofol may alter strain-induced endothelin-1 (ET-1) secretion and nitric oxide production, and to identify the putative underlying signaling pathways in human umbilical vein endothelial cells.**

Methods: **Cultured human umbilical vein endothelial cells were exposed to cyclic strain in the presence of propofol, and ET-1 expression was examined by Northern blotting and enzyme-linked immunosorbent assay kit. Activation of extracellular signal-regulated protein kinase, endothelial nitric oxide synthase, and protein kinase B were assessed by Western blot analysis.**

Results: **The authors show that propofol inhibits strain-induced ET-1 expression, strain-increased reactive oxygen species formation, and extracellular signal-regulated protein kinase phosphorylation. On the contrary, nitric oxide production, endothelial nitric oxide synthase activity, and protein kinase B phosphorylation were enhanced by propofol treatment. Furthermore, in the presence of PTIO, a nitric oxide scavenger, and KT5823, a specific inhibitor of cyclic guanosine monophosphate-dependent protein kinase, the inhibitory effect of propofol on strain-induced extracellular signal-regulated protein kinase phosphorylation and ET-1 release was reversed.**

Conclusions: **The authors demonstrate for the first time that propofol inhibits strain-induced ET-1 secretion and enhances strain-increased nitric oxide production in human umbilical vein endothelial cells. Thus, this study delivers important new insight into the molecular pathways that may contribute to the proposed hypotensive effects of propofol in the cardiovascular system.**

PROPOFOL has been used as an anesthetic agent for more than 20 yr; alternatively, it has been registered for use as a sedative in the intensive care unit setting because it is easy to administer and monitor, and also because of its simple onset and offset.[1,2,3] It may cause a certain degree of peripheral vasodilatation, which is related to the nitric oxide pathway involvement,[4–7] but the mechanism of its cardiovascular effect appears to be complicated and is only partially understood, even after more than 20 yr of research. Propofol possesses an antioxidative effect,[7] and immunomodulation[8] implies that it may have potential benefits on cardiovascular diseases.

Among the earliest indications of vascular dysfunction is an impaired regulation of vasomotion, representing disturbed homeostasis of vascular cells.[9] Key regulators of vasomotor function are the vasodilator nitric oxide and the vasoconstrictor endothelin-1 (ET-1).[10,11] In particular, endothelial nitric oxide exerts vasoprotective effects.[10] Its deficiency is associated with increased cardiovascular risks in pathologic situations such as diabetes, metabolic syndrome, hypertension, and atherosclerosis.[10] In addition, among the endogenous mediators of cardiovascular disorders, ET-1, a 21-amino-acid peptide, is a primary antecedent in coronary heart disease.[12–15] Such effects are mediated by extremely potent vasopressors and mitogenic responses for ET-1 in the vasculature.[16,17] Results from preclinical studies in humans, as well as in animal studies, showed that plasma ET-1 levels are consistently elevated in many spasm-related cardiovascular diseases,[16] and that blockers for ET receptors can substantially alleviate complications of such diseases.[16,17]

Under physiologic conditions, the highly controlled release of low amounts of nitric oxide by constitutive nitric oxide synthase in vascular endothelium (eNOS) dilates blood vessels.[10] In addition; endothelial cells are constantly under the influence of mechanical forces, including cyclic strain, as a consequence of vessel contraction and relaxation. Recently, numerous studies have shown that oxidative stress, represented by reactive oxygen species (ROS), is capable of significantly altering vascular function.[18–20] Previous studies reported that intracellular ROS levels are elevated in endothelial cells after cyclic strain treatment.[21–23] We have demonstrated that intracellular ROS mediate cyclic strain-induced ET-1 expression *via* the Ras/Raf/extracellular signal-regulated kinase (ERK) signaling pathway.[23]

However, no existing studies address the interference of propofol on ET-1 expression and nitric oxide production in vascular endothelial cells. The present study aims to investigate the effect of propofol on nitric oxide production and strain-induced ET-1 expression, and to identify the signaling protein kinase cascades that may be responsible for the putative effect of propofol.

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Materials and Methods

Materials

This study was approved by the Research Ethics Committee of China Medical University (Taichung, Taiwan). Imubind ET-1 enzyme-linked immunosorbent assay kits were purchased from Amersham-Pharmacia (Amersham, United Kingdom). 2′,7′-dichlorofluorescin diacetate was obtained from Serva Co. (Heidelberg, Germany). Propofol was obtained from B. Braun (Melsungen, Germany), and stock solutions of up to 1 mM propofol in phosphate-buffered saline (PBS) were prepared by diluting 100 mM propofol (dissolved in 100% dimethylsulfoxide). All other chemicals of reagent grade were obtained from Sigma-Aldrich (St. Louis, MO).

Endothelial Cell Culture

Human umbilical vein endothelial cells (HUVECs) were obtained from PromoCell (Heidelberg, Germany) as cryopreserved cells. After thawing, the cells were plated into cultured flasks and cultured to confluence in endothelial cells medium (MCDB 131 medium, PromoCell) containing hydroxyethylpiperazine ethanesulfonic acid (28 mM), fetal calf serum (2%), human recombinant epidermal growth factor (0.1 ng/ml), human recombinant basic fibroblast growth factor (1.0 ng/ml), gentamycin (50 μg/ml), amphotericin B (50 ng/ml), and synthetic hydrocortisone (1.0 μg/ml), and were supplemented with supplement mix (PromoCell) containing endothelial cell growth factor and heparin. Cells were grown at 37°C in a humidified 5% CO_2 atmosphere for 3 to 4 days. Confluent cultures between passages 2 and 10 were used for all experiments. The cultured HUVECs were randomly assigned to one of the following groups: Group 1 for ET-1 mRNA analysis: HUVECs were either controls or treated with different concentrations of propofol (1–30 μM) in the absence or the presence of strain treatment for 6 h. Group 2 for ET-1 secretion analysis: HUVECs were treated with propofol (1–30 μM) in the absence or the presence of strain treatment for 24 h. Group 3 for ROS detection: HUVECs were treated with propofol (1–30 μM) in the absence or the presence of strain treatment for 1 h, or HUVECs were either controls or treated with cyclic strain or hydrogen peroxide (H_2O_2, 25 μM) in the presence of propofol (30 μM), or antioxidant Trolox (200 μM) for 1 h. Group 4 for ERK phosphorylation analysis: HUVECs were treated with propofol (1–30 μM) in the absence or the presence of strain treatment for 30 min, or HUVECs were either controls or treated with cyclic strain or H_2O_2 (25 μM) in the presence of propofol (30 μM), or Trolox (200 μM) for 30 min. Group 5 for nitric oxide measurement: HUVECs were in control condition; treated with strain alone for 10, 30, 60, or 120 min; or treated with strain in the presence of propofol (30 μM) for 10, 30, 60, or 120 min; or HUVECs were either controls or treated with cyclic strain in the absence or presence of propofol (1–30 μM) for 60 min. Group 6 for eNOS and protein kinase B (Akt) phosphorylation analysis: HUVECs were in control condition; treated with strain alone for 5, 15, 30, or 60 min; or treated with strain in the presence of propofol (30 μM) for 5, 15, 30, or 60 min.

In Vitro *Cyclic Strain on Cultured Endothelial Cells*

Endothelial cells cultured on the flexible membrane base were subjected to cyclic strain produced by a computer-controlled application of sinusoidal negative pressure, as described previously.[23]

Northern Blot Analysis

Preparation of total RNA and Northern blot analyses of ET-1 and 18S RNA were performed as described previously.[23]

Measurement of ET-1 Concentration

ET-1 levels were measured in culture medium using a commercial enzyme-linked immunosorbent assay kit (Amersham-Pharmacia). Results were normalized to cellular protein content in all experiments, and expressed as a percentage relative to the cells incubated with the vehicle.

Detection of Intracellular ROS

Measurement of intracellular ROS formation in HUVECs was recorded by monitoring changes in dichlorofluorescein fluorescence as described previously.[22]

Western Blot Analysis

Western blot analysis was performed as described previously.[23] Membranes were blocked with 5% nonfat dry milk (NFDM) in PBS-0.1% Tween for 90 min and washed 3 times with PBS-0.1% Tween-1% NFDM, and then incubated with a 1:500 dilution of antiphospho-ERK antibodies (New England BioLabs; Beverly, MA), anti-ERK antibodies (Santa Cruz Biotechnology; Santa Cruz, CA), anti-Ser1177 phospho-eNOS antibodies, anti-Ser473 phospho-Akt antibodies, anti-Akt antibodies (Cell Signaling Technology; Beverly, MA); or anti-eNOS antibodies (BD Bioscience; San Jose, CA) in PBS-1% NFDM for 1 h and washed 3 times with PBS-0.1% Tween-1% NFDM; and then incubated with a 1:4,000 dilution of the horseradish peroxidase-coupled appropriate secondary antibody (Pierce; Rockford, IL) in PBS-0.1% Tween-1% NFDM and washed 3 times with PBS-0.3% Tween-1% NFDM.

Measurement of Nitrate/Nitrite Levels

The culture medium was stored at −70°C until use. After the medium had been thawed, the sample was deproteined with 2 volumes of 4°C 99% ethanol and centrifuged (3 000 g for 10 min). These medium samples (100 μl) were injected into a collection chamber containing 5% vanadium trichloride. This strong reducing environment converts

both nitrate and nitrite to nitric oxide. A constant stream of helium gas carried nitric oxide into a nitric oxide analyzer (Seivers 270B NOA; Seivers Instruments Inc.; Boulder, CO), where the nitric oxide was reacted with ozone, resulting in the emission of light. Light emission is proportional to the nitric oxide formed; standard amounts of nitrate were used for calibration.

Measurement of Nitric Oxide Synthase Activity

To measure nitric oxide synthase (NOS) activity, L-arginine to L-citrulline conversion was assayed in cells with a NOS detection assay kit (Calbiochem; San Diego, CA) according to the manufacturer's instructions. Briefly, cells were lysed with a buffer containing 1% NP-40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate. After incubation on ice for 30 min, cell extracts were centrifuged to remove cell debris. Protein extracts were then incubated for 60 min at 37°C in a solution of 10 μM L-[^{3}H]arginine, 1 mM nicotinamide adenine dinucleotide phosphate, 1 μM flavin adenine dinucleotide, 1 μM flavin mononucleotide, 100 nM calmodulin, 600 μM $CaCl_2$, and 3 μM tetrahydrobiopterin in a final volume of 40 μl. The reaction was stopped by the addition of 400 μl of stop buffer (10 mM EDTA, 50 mM HEPES buffer, pH 5.5) to the reaction mixture. Then 100 μl of equilibrated resin was added to each mixture. Reaction samples were transferred to spin cups and centrifuged at 10,000 g for 30 s. The radioactivity of the flow-through was measured by liquid scintillation counting. Enzyme activity was expressed as citrulline production in pmol · min · mg^{-1} · $protein^{-1}$.

Statistical Analysis

Data are presented as mean ± SEM. Statistical analysis was performed as appropriate using Student's *t* test or ANOVA, followed by a Dunnett multiple comparison test using Prism version 3.00 for Windows (GraphPad Software; San Diego, CA). *P* values < 0.05 were considered to be statistically significant.

Results

Effect of Propofol on Strain-induced ET-1 Expression in Endothelial Cells

HUVECs cultured on flexible membrane bases were subjected to deformation to produce an average strain of 20%. We examined the effect of propofol on strain-increased ET-1 mRNA levels. HUVECs under cyclic strain for 6 h showed a significant increase in ET-1 mRNA levels (203.8 ± 31.2% over controls); however, treatment with propofol (3–30 μM) significantly reduced the strain-induced ET-1 expression (41.6 ± 13.5, 52.5 ± 12.5, and 56.6 ± 10.7% reduction with 3, 10, and 30 μM propofol, respectively) (fig. 1A). We then examined the effect of propofol on strain-increased ET-1 secretion. ET-1 released into the culture media was measured. As shown in figure 1B, HUVECs under cyclic strain for 24 h increased ET-1 secretion (112.3 ± 11.2% over controls), and also, propofol (3–30 μM) significantly inhibited strain-increased ET-1 secretion (34.8 ± 12.4, 45.8 ± 7.5, and 52.9 ± 8.7% reduction with 3, 10, and 30 μM propofol, respectively). These data indicate that propofol inhibits strain-increased ET-1 secretion in endothelial cells.

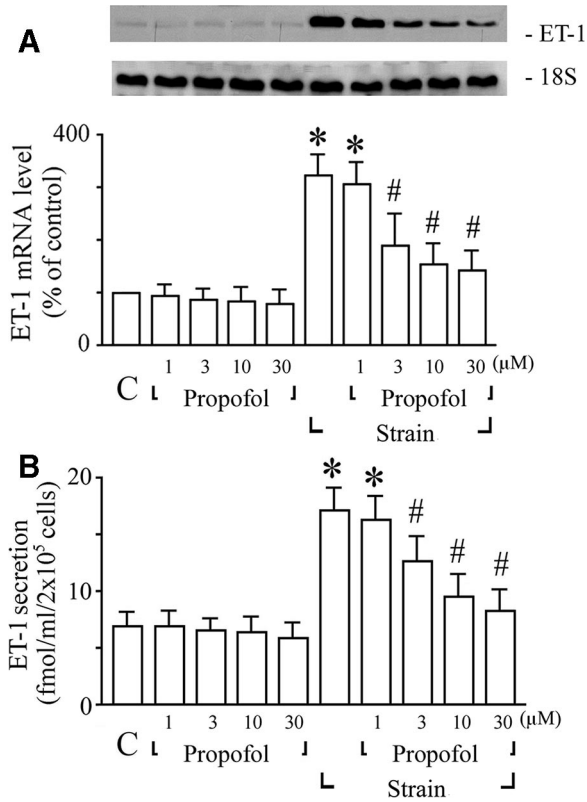

Fig. 1. Effects of propofol on strain-induced endothelin-1 (ET-1) expression. (*A*) Propofol inhibits strain-induced ET-1 mRNA expression. Human umbilical vein endothelial cells (HUVECs) were either vehicle controls (C, lane 1), or treated with different concentrations of propofol (1–30 μM) in the absence (lanes 2–5) or the presence of strain treatment (lanes 6–10) for 6 h. Total RNA was extracted and Northern hybridization was performed with 32P-labeled ET-1 as the probe. 18S RNA was used to normalize the RNA applied in each lane. Data are presented as percentage changes of experimental groups, as compared with untreated controls. (*B*) Propofol inhibits strain-induced ET-1 secretion. HUVECs were treated with propofol (1–30 μM) in the absence or the presence of strain treatment. After strain (20%) for 24 h, the culture media were collected and the concentrations analyzed by enzyme immunoassay. Results are presented as mean ± SEM (n = 6). * *P* < 0.05 *versus* unstrained control cells. # *P* < 0.05 *versus* strained cells (analysis of variance [ANOVA]).

Propofol Inhibited Strain-increased ROS Formation

Our previous studies showed that cyclic strain increases ROS formation in endothelial cells, which is involved in ET-1 induction.[22,23] We next examined whether propofol prevents the strain-increased ROS formation. HUVECs were treated with propofol (1–30 μM)

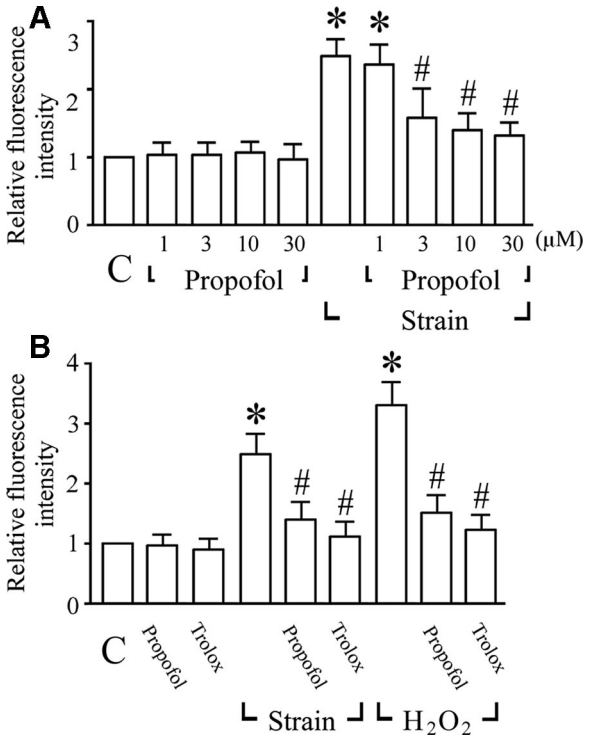

Fig. 2. Effects of propofol on strain-increased reactive oxygen species (ROS) formation. (*A*) Effect of propofol (1–30 μM) on strain-induced ROS generation. (*B*) Human umbilical vein endothelial cells (HUVECs) from either control (C) or treated with cyclic strain or H_2O_2 (25 μM) in the presence of propofol (30 μM), or antioxidant Trolox (200 μM) for 1 h. Fluorescence intensities of cells are shown as relative intensity of experimental groups, as compared with untreated control cells. The results show mean ± SEM (n = 6). **P* < 0.05 *versus* control; #*P* < 0.05 *versus* strain- (or H_2O_2-) treated cells (analysis of variance [ANOVA]).

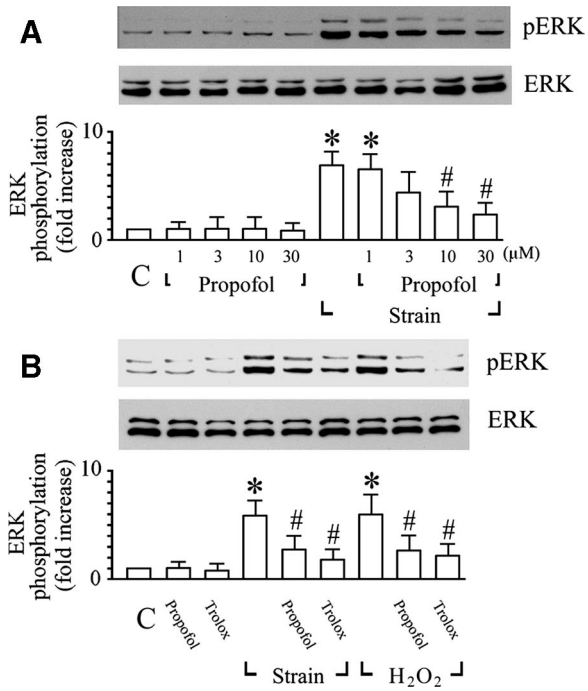

Fig. 3. Inhibitory effects of propofol on strain-increased extracellular signal-regulated kinases (ERK) phosphorylation. (*A*) Effect of propofol (1–30 μM) on strain-activated ERK phosphorylation. (*B*) Effect of propofol on strain- or H_2O_2-induced phosphorylation of ERK. Human umbilical vein endothelial cells (HUVECs) were incubated with either propofol (30 μM) or antioxidant Trolox (200 μM) and stimulated with cyclic strain or H_2O_2 (25 μM) for 30 min. Data are represented as fold increase relative to control groups. The results show mean ± SEM (n = 6). **P* < 0.05 *versus* control; #*P* < 0.05 *versus* strain (or H_2O_2) alone (analysis of variance [ANOVA]).

in the absence or presence of strain treatment. The addition of propofol (3–30 μM) to cultured HUVECs significantly inhibited strain-induced ROS formation, as measured after strain treatment for 1 h (fig. 2A). The treatment of propofol (10 μM) or antioxidant Trolox (200 μM) to cultured HUVECs also significantly inhibited strain- or H_2O_2-induced ROS formation (fig. 2B). These findings clearly demonstrate that propofol inhibits strain-increased intracellular ROS levels in endothelial cells.

Propofol Inhibited Strain-activated ERK Phosphorylation in Endothelial Cells

To gain insight into the mechanism of action of propofol, we thus examined whether propofol affects intracellular protein kinase signaling pathways. Given that the ERK signaling pathway is involved in strain-induced ET-1 expression,[22,23] we further investigated whether propofol inhibits the ERK pathway in strain-treated endothelial cells. We examined the phosphorylation of ERK in HUVECs exposed to propofol (1–30 μM) in the absence or presence of strain treatment. As shown in figure 3, HUVEC exposure to strain treatment for 30 min rapidly activated phosphorylation of ERK. However, HUVECs treated with propofol (10 and 30 μM) showed significantly decreased strain-induced ERK phosphorylation (fig. 3A). Moreover, HUVECs treated with propofol (10 μM) or Trolox (200 μM) also showed significantly decreased strain- or H_2O_2-induced ERK phosphorylation (fig. 3B). These findings imply that propofol inhibits strain-activated ERK signaling pathways *via* attenuation of ROS formation in endothelial cells.

Effects of Propofol on Nitric Oxide Synthesis, NOS Activity, Phospho-eNOS, and Phospho-Akt in HUVECs Under Cyclic Strain

To determine propofol's mechanisms of action, NOS, NOS activity, phosphor-eNOS, and phospho-Akt (for serine 473 and threonine 308 isoforms) were detected. Exposure of HUVECs to propofol time- and dose-dependently enhanced the strain increase of nitric oxide generation (fig. 4, A and B). All three NOS isoforms characterized to date depend on calmodulin activation, but are distinguished from each other by their calcium sensitivity and enzymatic activity. A constitutive NOS activity was detected and quantified in whole extracts of cells, and this activity was dependent on the presence of calcium (fig. 4C). Exposure of HUVECs to propofol (10 and 30 μM) also enhanced the strain-increased NOS ac-

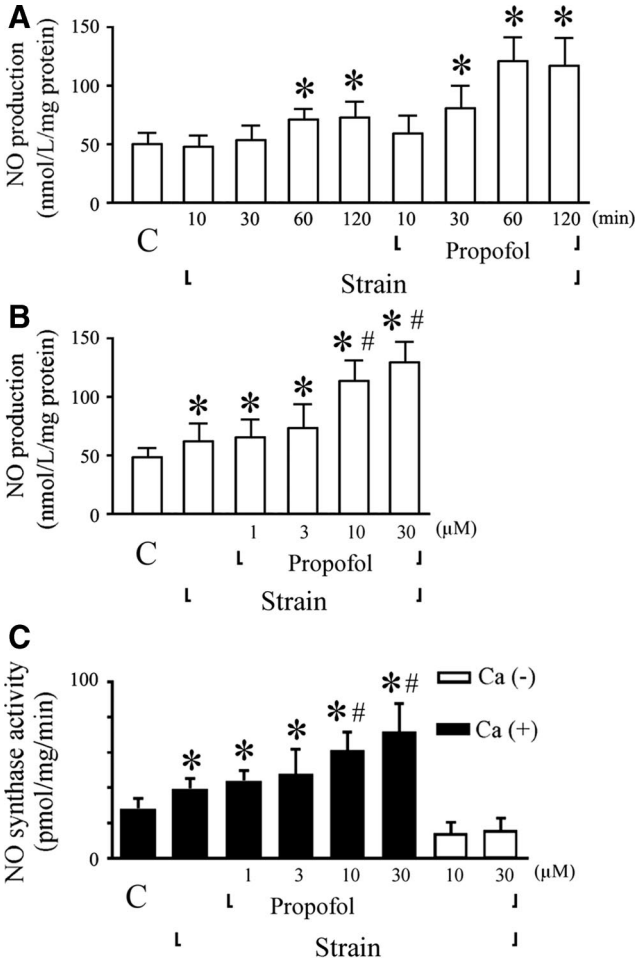

Fig. 4. Propofol increases nitric oxide (NO) production and NO synthase (NOS) activity in Human umbilical vein endothelial cells (HUVECs). (*A*) Time course of propofol-induced NO production in HUVECs. Cells were in control condition, treated with propofol (30 μM) for 10, 30, 60, or 120 min. (*B*) Cells from either control or treated with cyclic strain in the absence or presence of propofol (1–30 μM) for 60 min. (*C*) Cells were treated with propofol (1–30 μM) for 60 min. NOS activities were measured by citrulline formation from cell lysate incubated with (black bars) or without Ca^{2+} (white bars). NOS activity in the absence of Ca^{2+} indicates inducible NOS activity, whereas its activity in the presence of Ca^{2+} represents constitutive NOS activity. Results are shown as mean ± SEM (n = 6). * *P* < 0.05 *versus* control. # *P* < 0.05 *versus* strain-alone treated cells (analysis of variance [ANOVA]).

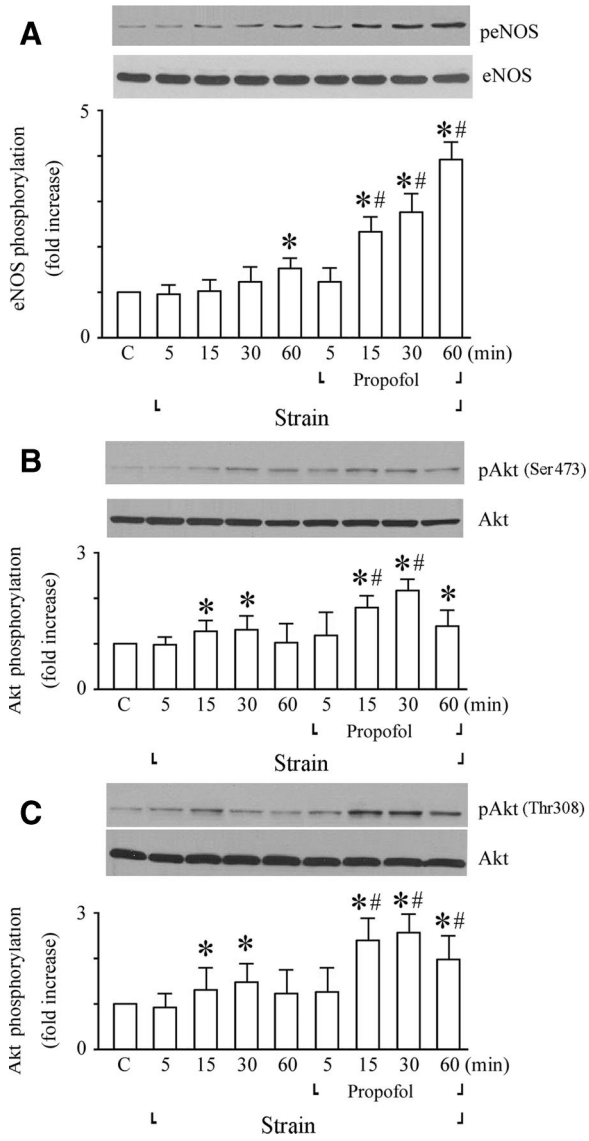

Fig. 5. Propofol enhanced cyclic strain-increased phospho-nitric oxide synthase in vascular endothelium (eNOS). (*A*) and phospho-protein kinase B (Akt) (*B*, *C*) in Human umbilical vein endothelial cells. Cells were treated and protein phosphorylations were detected by Western Blotting as described in Methods section. Propofol treatment, in the presence of cyclic strain, significantly enhanced eNOS phosphorylation and Akt (Ser 473 and Thr 308) phosphorylations as compared to strain-stimulated alone groups. Results are shown as mean ± SEM (n = 6). **P* < 0.05 *versus* control. #*P* < 0.05 *versus* strain-alone treated cells (analysis of variance [ANOVA]).

tivity at 60 min after stimulation (fig. 4C). In addition, as shown in figure 5, HUVEC exposure to strain treatment activated phosphorylation of eNOS and Akt. Propofol treatment in HUVECs under cyclic strain significantly enhanced strain-increased phospho-eNOS (fig. 5A) and phospho-Akt (Ser 473 and Thr 308) (fig. 5, B and C). Exposure of HUVECs to propofol (10 and 30 μM) alone induced increase of nitric oxide generation, NOS activity, and eNOS phosphorylation. Overall, the magnitude of the effect of propofol on HUVECS not subjected to strain on the NO production was weaker, as compared with propofol increase in nitric oxide production in HUVECs under strain treatment (data not shown).

Propofol Modulates Strain-induced ET-1 Release in Part via *Nitric Oxide/Cyclic Guanosine Monophosphate-dependent Protein Kinase Pathway*

To examine whether propofol antagonizes strain-induced ET-1 release through nitric oxide production and protein kinase (PKG) pathways, we used PTIO(100 μM), a nitric oxide scavenger, and KT5823 (1 μM), a specific inhibitor of PKG, to determine the involvement of nitric oxide/PKG pathway in propofol modulation of strain-induced ERK phosphorylation and ET-1 release. In the presence of PTIO(100 μM), or KT5823 (1 μM), the inhib-

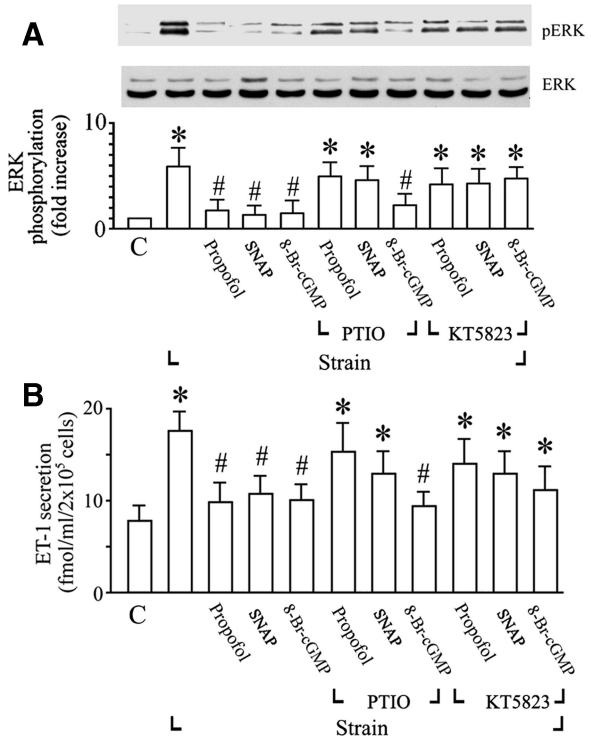

Fig. 6. Modulation of strain-increased extracellular signal-regulated kinase (ERK) phosphorylation and endothelin-1 (ET-1) release by propofol, in part *via* nitric oxide/cyclic guanosine monophosphate (cGMP)-dependent protein kinase (PKG) pathway. (*A*) Under strain treatment, cells were incubated with propofol (30 M), S-Nitroso-N-acetylpenicillamine (SNAP) or 8-bromoguanosine-3′,5′-cyclic monophosphate (100 μM) for 30 min in the absence or presence of PTIO (nitric oxide scavenger) or KT-5823 (a specific inhibitor of PKG; 1 μM). After strain treatment, cells were lysed and the phosphorylation of ERK was determined. Data are represented as fold induction relative to control groups. Results are shown as mean ± SEM (n = 5). * $P < 0.05$ *versus* control; # $P < 0.05$ *versus* strain treatment. (*B*) PTIO or KT5823 reversed the inhibitory effect of propofol on strain-induced ET-1 release in human umbilical vein endothelial cells (HUVECs). Cells were treated with cyclic strain for 24 h in the absence or presence of propofol, or propofol plus PTIO or KT-5823. Data were presented as a percentage change of experimental groups, as compared with untreated controls. Results are shown as mean ± SEM (n = 6). * $P < 0.05$ *versus* control; # $P < 0.05$ *versus* cyclic strain.

itory effect of propofol on strain-increased ERK phosphorylation and ET-1 release was reversed (fig. 6, A and B). In contrast, HUVECs exposed to cyclic strain in the presence of the nitric oxide donor S-Nitroso-N-acetylpenicillamine (100 μM) or cyclic guanosine monophosphate (cGMP) analogs, 8-bromoguanosine-3′,5′-cyclic monophosphate (100 μM), which activates PKG, showed significant inhibition of strain-stimulated ERK phosphorylation and ET-1 release. These results indicate the involvement of nitric oxide/PKG pathway in propofol modulation of strain-stimulated ET-1 release.

Discussion

The major new finding of this work is that propofol inhibits strain-induced ET-1 secretion and enhances nitric oxide production in endothelial cells. It is supported by the observations that propofol inhibits strain-induced ET-1 expression in part *via* the attenuation of ROS formation and the enhancement of nitric oxide production in endothelial cells under strain. Previous studies, including ours, have indicated that hemodynamic forces, including shear flow and pressure-induced strain,[22–25] can stimulate the ET-1 gene expression. Recent studies provide evidence that ROS may act as second messengers in cells exposed to various stimuli.[22–24] Previous studies from our collaborating laboratory and others have shown that cyclic-strain treatment of endothelial cells can induce intracellular ROS generation.[21–23] This ROS generation is sustained at an elevated level as long as the mechanical forces are maintained, and returns to basal levels with the removal of the forces.[21] Elevated ROS levels are involved in the release of ET-1,[22,23] and this gene induction can be attenuated by antioxidant pretreatment of cells.[22,23,26,27]

Some of the plant-derived agents possess antioxidative effects,[22,26–32] and may have potential therapeutic benefit in clinical use. The plant-derived propofol is a potent intravenous hypnotic agent commonly administered for induction and maintenance of anesthesia, and for sedation in the intensive care unit.[1-3] It is also a potent antioxidant.[3,7,8] The results of our present study further demonstrated that propofol reduced the strain-induced ROS generation in the endothelial cells. In particular, it has been demonstrated that activation of ERK is redox-sensitive,[21–23] and that suppression of ROS inhibits strain-induced ET-1 gene expression.[22,23] One possible explanation for the inhibitory effect of propofol on strain-induced ET-1 expression may thus be its ability to attenuate ROS formation. Alternatively, propofol may inhibit strain-induced ET-1 gene expression by virtue of its increasing nitric oxide production.[5,7] We found that propofol treatment, in the absence of cyclic strain, enhances the production and release of nitric oxide from HUVECs (data not shown). The magnitude of the effect of propofol on HUVECS not subjected to strain on the nitric oxide production was weaker, as compared with propofol increase in nitric oxide production in HUVECs under strain treatment. This is similar in nature to previous reports that application of propofol alone stimulates the production of nitric oxide from cultured vascular endothelial cells.[33,34] Our findings also indicate that propofol *via* nitric oxide production suppresses the strain-stimulated ERK activation and ET-1 expression. Nitric oxide acting through soluble guanylyl cyclase and cGMP formation is a negative regulator of ET-1 gene induction.[10,35–37] Our findings further support that nitric oxide acts as a negative regulator in strain-induced ET-1 expression under propofol treatment. We also demonstrated that HUVEC exposure to strain treatment activated phosphorylation of eNOS and Akt. Nitric oxide synthesized by eNOS is regarded as an endothelial cell survival factor.[38] The serine-threonine kinase Akt activation has been reported to activate eNOS, which leads to nitric oxide production, pro-

moting cell survival. The Akt/eNOS/nitric oxide pathway has been identified as an important survival pathway in endothelial cells.[39,40] Previously, Wang *et al.* reported that propofol treatment, in the presence of H_2O_2, significantly increased eNOS expression but not Akt phosphorylation.[41] In contrast, in the present study, we found that propofol treatment in HUVECs under cyclic strain significantly enhanced strain-increased phospho-eNOS and phospho-Akt. In addition, HUVECs exposed to cyclic strain in the presence of the nitric oxide donor S-Nitroso-N-acetylpenicillamine or cGMP analogs, 8-bromoguanosine-3, 5′-cyclic monophosphate, which activates PKG, showed significant inhibition of strain-stimulated ERK phosphorylation and ET-1 release. These results further suggest the involvement of nitric oxide/PKG pathways in propofol modulation of strain-stimulated ET-1 release.

In conclusion, the data obtained in the present study suggest that the propofol-induced nitric oxide production and suppression of cyclic strain-induced ET-1 expression can be considered as one of the mechanisms responsible for the protective effect of propofol in vascular vessels. These findings have highlighted the therapeutic potentials of using plant-derived propofol for the treatment of arteriosclerosis and hypertension. In light of our findings, propofol can be considered as a potentially active compound for use in conditions where ROS are implicated, although clinical studies are needed to establish the usefulness of this natural product in the treatment or prevention of human diseases.

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