Regular Article

Abnormal Pressure Stress Reduces Interleukin-1 β -Induced Cyclooxygenase-2 Expression in Cultured Rat Vascular Smooth Muscle Cells

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Elevated mechanical stress on blood vessels associated with hypertension has a direct effect on the function of vascular endothelial cells and vascular smooth muscle cells (VSMCs). In the present study, we have identified the effect of pulsatile pressure stress on cyclooxygenase-2 (COX-2) expression induced by interleukin (IL)-1 β in cultured rat VSMCs. VSMCs were isolated from aortic media of Wistar rats and cultured. Pulsatile pressure applied to VSMCs was repeatedly given between either 80 and 160 mmHg, which simulates systolic hypertension, or 80 and 120 mmHg, which simulates normal blood pressure, at a frequency of 4 cycles per min using our original apparatus. Pressure loading that simulates systolic hypertension reduced IL-1 β -induced COX-2 expression. The pressure also inhibited the rapid and transient phosphorylation of extracellular signal-regulated kinase (ERK) induced by IL-1 β . IL-1 β -induced COX-2 expression was significantly inhibited by a specific conventional protein kinase C (PKC) inhibitor. Pressure loading that simulates systolic hypertension also reduced phorbol myristate 13-acetate (PMA) (a PKC activator)-induced COX-2 expression and the rapid and transient phosphorylation of ERK. Pressure loading that simulates normal blood pressure had no effect on IL-1 β - and PMA-induced COX-2 expression. The present study shows that pressure stress between 80 and 160 mmHg, which simulates systolic hypertension reduces IL-1 β -induced COX-2 expression by affecting a mechanism involving PKC and ERK signaling pathways. Downregulation of COX-2 expression in VSMCs by abnormal pressure stress may further worsen local vascular injury associated with hypertension.

Key words pulsatile pressure stress; cyclooxygenase-2; interleukin-1 β ; extracellular signal-regulated kinase; protein kinase C; vascular smooth muscle cell

INTRODUCTION

Vascular endothelial cells and vascular smooth muscle cells (VSMCs) are continuously exposed to not only to biological substances and nerve stimulation but also to mechanical stress associated with blood flow and pulsation. Elevated mechanical stress to vascular walls associated with high pressure modulates vascular remodeling, and certainly plays a role in the pathogenesis of atherosclerosis.¹⁾ The mechanical stress applied to vessel walls can be distinguished into three types, *i.e.*, stretch, shear stress, and pressure. Although shear stress due to blood flow is unlikely to be directly affected on VSMCs, pulsatile stretch and pressure due to pulsatile change of blood pressure are directly loaded on VSMCs.

Cyclooxygenase (COX) is a key enzyme catalyzing the rate-limiting step in synthesis of prostaglandin. COX-1 is constitutively expressed in most cells, whereas COX-2 is normally undetectable but is inducible under circumstances such as inflammation with stimulation by variety of cytokines or vasoactive substances. Normal range, *i.e.*, healthy state, of fluid shear stress as well as cyclic stretch are known to induce COX-2 in vascular endothelial cells,^{2,3)} suggesting these

mechanical stress-induced COX-2 expressions have a role for a general housekeeping function because prostacyclin (PGI₂) is predominantly produced by COX in endothelial cells. PGI₂ in vasculature has potent anti-inflammatory, antiplatelet, anti-proliferative and vasodilatory effect.⁴⁾ PGI₂ is also produced by COX in VSMCs as the major prostanoid.⁵⁾ Cytokine, such as interleukin (IL)-1 β -induced COX-2 expression in VSMCs is thought to function primarily as a defensive and compensatory mechanism for endothelial dysfunction at a local vascular injury site.⁶⁾ However, effect of pressure stress on COX-2 expression in VSMCs has not been characterized.

We have developed a pulsatile pressure-loading apparatus that can simulate various types of hypertension in cultured cells.⁷⁾ By using this apparatus, we found that pulsatile pressure that simulates systolic hypertension, suppresses the IL-1 β -induced expression of inducible nitric oxide synthase (iNOS).⁸⁾ The mechanism of iNOS suppression by pressure stress involves inhibition of the activation of extracellular signal-regulated kinase (ERK) at the late stage, that is, persistent activation that occurs after 1 h of IL-1 β stimulation.⁸⁾ IL-1 β is also known to activate ERK at a transient phase peaking at 20–30 min, that is, at the early stage.^{9,10)} But the effect of pulsatile pressure on IL-1 β -induced ERK activation at the early stage has not been elucidated. The signaling cascade of COX-2 expression in most cells has been well characterized, and the

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protein kinase C (PKC)- and mitogen-activated protein kinase (MAPK)-mediated signaling cascade is believed to be an important cascade leading to COX-2 induction in VSMCs.¹¹⁾ In fact, phorbol-12-myristate-13 acetate (PMA), a PKC activator, has been shown to induce MAPK as well as COX-2 expression in many cell types including VSMCs.^{12,13)} However, the role of PKC and MAPK for COX-2 expression seems somewhat controversial, with depending on different stimulators and/or different cell types.^{13–17)}

In the present study, therefore, we investigated the effect of pulsatile pressure stress between 80 and 160 mmHg, which simulates systolic pressure elevation as one of the most common clinical observations of hypertension, on IL-1 β - and PMA-induced COX-2 expression as well as transient ERK activation. Based on our results, we propose that pressure stress between 80 and 160 mmHg reduces IL-1 β -induced COX-2 expression by affecting a mechanism involving PKC and ERK signaling pathways.

MATERIALS AND METHODS

Cell Culture VSMCs were enzymatically isolated from aortic media obtained from 6–7-week-old Wistar rats using collagenase and elastase.¹⁸⁾ Cells were then cultured until confluence, as previously described.¹⁸⁾ Primary VSMCs were used throughout the experiments in each figure refers to individual studies on separate primary cultures. At 24 h before the experiments, the medium was replaced with a serum-free medium containing 0.1% bovine serum albumin to allow the cells to quiesce. Cells were then treated with IL-1 β or PMA. In some experiments, VSMCs were treated with inhibitors of either MAPK or PKC for 1 h before IL-1 β or PMA stimulation. This study was conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals by the Animal Research Committee of Health Sciences University of Hokkaido, Japan.

Application of Pressure Stress An original design pulsatile pressure-loading apparatus was utilized in the experiments, as described previously.⁸⁾ In brief, culture dishes were placed in the pressure-loading apparatus, and VSMCs were exposed to pulsatile atmospheric pressure either between 80 and 160 mmHg, which simulates systolic hypertension, or between 80 and 120 mmHg, which simulates normal blood pressure, at a rate of 4 cycles/min. IL-1 β or PMA was added to the culture medium at the beginning of pressurization.

Western Blot Analysis Protein was isolated from VSMCs using buffers and protease inhibitors. Lysate protein was separated out by electrophoresis on either 7.5 or 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel and transferred to a polyvinylidene difluoride (PVDF) membrane as described previously.¹⁵⁾ The blot was incubated for 2 h with a primary antibody. Subsequently, the immunoblot was incubated with a horseradish peroxidase-conjugated secondary antibody and visualized using an enhanced chemiluminescence kit (Millipore, Billerica, MA, U.S.A.). Anti- α -tubulin antibody and anti-total ERK antibody were used as internal controls for COX-2 and phosphorylated ERK, respectively. All bands were analyzed by densitometry using ImageJ software.

Real-Time RT-PCR Total RNA was extracted from cultured VSMCs using the TRI reagent[®] (Sigma-Aldrich, St. Louis, MO, U.S.A.) according to the manufacturer's instructions. Real-time RT-PCR was carried out using a 7500

Real-Time PCR system (Applied Biosystems, Foster City, CA, U.S.A.) and a SuperScript III Platinum SYBR Green One-Step qRT-PCR Kit (Invitrogen, Carlsbad, CA, U.S.A.), as described previously.¹⁵⁾ The primer sequences were also described previously.¹⁵⁾ β -actin mRNA expression was used as the internal control for COX-2 mRNA. The relative expressions of individual targets of mRNA were calculated by $\Delta\Delta^{CT}$ method.

Materials Fetal calf serum, penicillin, and streptomycin were obtained from Life Technologies (Grand Island, NY, U.S.A.). IL-1 β was from BD Biosciences (Bedford, MA, U.S.A.). PMA was from Biomol (Plymouth Meeting, PA, U.S.A.). Anti-COX-2 polyclonal goat antibody was from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.). Anti- α -tubulin rabbit polyclonal antibody was from Medical & Biological Laboratories (Aichi, Japan). Bisindolylmaleimide I, Gö 6976, SCH 772984, SB 203580, SP 600125, anti-phosphorylated and total ERK antibodies were from Calbiochem (San Diego, CA, U.S.A.). Horseradish peroxidase-conjugated secondary antibodies were from Invitrogen. The other reagents used in this study were of special grade and purchased from local suppliers unless otherwise noted.

Statistical Analysis Data were presented as mean $\pm$ standard error (S.E.) values of replicate experiments. Statistical analysis was conducted with a two-tailed Student's *t*-test or one-way ANOVA followed by Dunnett's test for multiple comparisons, or two-way ANOVA followed by Tukey's test for multiple comparisons. Significant difference was considered at *p*-values less than 0.05.

RESULTS

Pulsatile Pressure between 80–160 mmHg Reduces IL-1 β -Induced COX-2 Expression Pulsatile pressure of 80–160 mmHg suppressed 3 ng/mL IL-1 β -induced COX-2 protein expression in a time-dependent manner, in particular at 24 h (Fig. 1A). When VSMCs were stimulated with 3, 10, or 30 ng/mL IL-1 β for 24 h, COX-2 protein expression was induced in a concentration-dependent manner, and pulsatile pressure suppressed this IL-1 β -induced COX-2 protein expression (Fig. 1B). Pulsatile pressure significantly suppressed IL-1 β -induced COX-2 protein and mRNA expression at 24 h (Figs. 1C, D). Pulsatile pressure itself induced neither COX-2 protein nor mRNA expression (Figs. 1B–D). Pulsatile pressure of 80–120 mmHg had no effect on IL-1 β -induced COX-2 protein and mRNA expression at 24 h (Figs. 2A, B).

Pulsatile Pressure between 80–160 mmHg Reduces IL-1 β -Induced Transient ERK Phosphorylation IL-1 β -induced COX-2 protein expression was almost completely inhibited by SCH 772984 (1 μ M), an inhibitor of ERK, but not by either SB 203580 (1 μ M), an inhibitor of p38 MAPK, or SP 600125 (1 μ M), an inhibitor of c-Jun N-terminal kinase (JNK) under non-pressurized condition (Fig. 3A). Therefore, we next examined the effect of pressure stress on IL-1 β -induced ERK phosphorylation. IL-1 β caused the rapid phosphorylation of ERK with a peak at 5–20 min after stimulation with IL-1 β (Fig. 3B). At 5 min after stimulation with IL-1 β , pulsatile pressure of 80–160 mmHg significantly suppressed ERK phosphorylation (Figs. 3B, C).

Conventional PKC Pathway Is Involved in IL-1 β -Induced COX-2 Expression Bisindolylmaleimide I acts as a competitive inhibitor of the ATP binding site of PKC, and shows a de-

Fig. 1. Effect of the Pulsatile Atmospheric Pressure of 80–160 mmHg on IL-1 β -Induced COX-2 Expression in Rat VSMCs

The cells were treated with 3 ng/mL of IL-1 β for the indicated time periods (A) or for 24 h (B–D) in the absence or the presence of pressure stress of 80–160 mmHg. COX-2 protein and COX-2 mRNA expression were analyzed by Western blotting and quantitative RT-PCR, respectively. COX-2 protein and mRNA expression was normalized by α -tubulin and β -actin, respectively. A and B: The representative protein expression of blotting images of COX-2 and α -tubulin. C and D: Summary of analysis expressed by taking samples treated with IL-1 β under non-pressurized conditions as 1. Bars show mean $\pm$ S.E. values ($n = 6$). ** $p < 0.01$ vs. IL-1 β under non-pressurized conditions.

gree of isozyme specificity, with *in vitro* IC₅₀ values ranging from nanomolar concentration for conventional and novel PKC to 5 μ M for all PKC isoforms including the atypical PKC.¹⁹⁾ IL-1 β -induced COX-2 protein expression was almost completely inhibited at 2 and 10 μ M of bisindolylmaleimide I with or without pulsatile pressure (Figs. 4A, B). Furthermore, Gö 6976 (2 μ M), a specific conventional PKC inhibitor, also completely inhibited the COX-2 expression (Fig. 4C).

Fig. 2. Effect of the Pulsatile Atmospheric Pressure of 80–120 mmHg on IL-1 β -Induced COX-2 Expression in Rat VSMCs

The cells were treated with 3 ng/mL of IL-1 β for 24 h in the absence or the presence of pressure stress of 80–120 mmHg. COX-2 protein (A) and COX-2 mRNA (B) expression were analyzed by Western blotting and quantitative RT-PCR, respectively. COX-2 protein and mRNA expression was normalized by α -tubulin and β -actin, respectively. A: Upper panel: representative blotting image of expressions of COX-2 and α -tubulin. Lower panel: Summary of densitometric analysis expressed by taking samples treated with IL-1 β under non-pressurized conditions as 1. B: Summary of analysis expressed by taking samples treated with IL-1 β under non-pressurized conditions as 1. Bars show mean $\pm$ S.E. values ($n = 6$).

Pulsatile Pressure between 80–160 mmHg Reduces PMA-Induced COX-2 Expression Pulsatile pressure of 80–160 mmHg suppressed 10 nM PMA-induced COX-2 protein expression in a time-dependent manner, in particular at 1 h (Fig. 5A). Pulsatile pressure significantly suppressed PMA-induced COX-2 protein expression at 1 h (Fig. 5B). Because mRNA expression is induced prior to protein expression, we next examined the effect of pulsatile pressure on PMA-induced COX-2 mRNA at 30 min. As a result, pulsatile pressure also significantly suppressed the COX-2 mRNA expression (Fig. 5C). Pulsatile pressure of 80–120 mmHg had no effect on IL-1 β -induced COX-2 protein expression at 1 h (Fig. 5D).

Pulsatile Pressure between 80–160 mmHg Reduces PMA-Induced Transient ERK Phosphorylation PMA-induced COX-2 protein expression was almost completely inhibited by SCH 772984 (1 μ M), SB 203580 (1 μ M) and SP 600125 (1 μ M) under non-pressurized condition (Fig. 6A). PMA caused the rapid phosphorylation of ERK with a peak at 5 min after stimulation with PMA (Fig. 6B). At 5 min after stimulation with PMA, pulsatile pressure of 80–160 mmHg significantly suppressed ERK phosphorylation (Figs. 6B, C).

Fig. 3. Suppressive Effect of the Pulsatile Atmospheric Pressure of 80–160 mmHg on IL-1 β -Induced Transient ERK Activation in Rat VSMCs

A: The cells were either pretreated with or without 1 μ M SCH772984, 1 μ M SB 203580 or 1 μ M SP 600125 for 1 h, and then treated with 3 ng/mL IL-1 β for 24 h. B and C: The cells were treated with IL-1 β for the indicated time periods (B) or 5 min (C) in the absence or the presence of the pressure stress of 80–160 mmHg. COX-2 protein expression and ERK activation were analyzed by Western blotting. COX-2 protein and phosphorylated ERK expression were normalized by α -tubulin and total ERK, respectively. A: Upper panel: representative blotting image of expressions of COX-2 and α -tubulin. Lower panel: Summary of densitometric analysis expressed by taking samples treated with IL-1 β as 1. B: The representative protein expression of blotting images of COX-2 and α -tubulin, and phosphorylated and total ERK, respectively. C: Upper panel: representative blotting image of expressions of phosphorylated and total ERK. Lower panel: Summary of densitometric analysis expressed by taking samples treated with IL-1 β under non-pressurized conditions as 1. Bars show mean $\pm$ S.E. values ($n=6$ for A, 7 for C). *** $p<0.001$, * $p<0.05$ vs. IL-1 β under non-pressurized conditions.

DISCUSSION

This study demonstrated that pulsatile pressure stress be-

Fig. 4. Effect of PKC Inhibitors on IL-1 β -Induced COX-2 Expression in Rat VSMCs

The cells were either pretreated with or without 10 μ M bisindolylmaleimide I (A), 2 μ M bisindolylmaleimide I (B) or 2 μ M G6 6976 (C) for 1 h, and then treated with 3 ng/mL IL-1 β for the 24 h in the absence or the presence of the pressure stress of 80–160 mmHg. COX-2 protein expression was analyzed by Western blotting. COX-2 protein expression was normalized by α -tubulin. A: The representative protein expression of blotting images of COX-2 and α -tubulin. B and C: Upper panel: representative blotting image of expressions of COX-2 and α -tubulin. Lower panel: Summary of densitometric analysis expressed by taking samples treated with IL-1 β under non-pressurized conditions as 1. Bars show mean $\pm$ S.E. values ($n=4$). *** $p<0.001$, ** $p<0.01$ vs. IL-1 β under non-pressurized conditions.

tween 80 and 160 mmHg, which simulates systolic pressure elevation as one of the most common clinical observations of hypertension, suppressed IL-1 β -induced COX-2 expression in VSMCs. Our findings also indicate that the suppressive effect

Fig. 5. Effect of the Pulsatile Atmospheric Pressure of 80–160 or 80–120 mmHg on PMA-Induced COX-2 Expression in Rat VSMCs

The cells were treated with 10 nM PMA for the indicated time periods (A), 1 h (B, D), and 30 min (C) in the absence or the presence of pressure stress of either 80–160 mmHg (A–C) or 80–120 mmHg (D). COX-2 protein and COX-2 mRNA expression were analyzed by Western blotting and quantitative RT-PCR, respectively. COX-2 protein and mRNA expression was normalized by α -tubulin and β -actin, respectively. A: The representative protein expression of blotting images of COX-2 and α -tubulin. B and D: Upper panel: representative blotting image of expressions of COX-2 and α -tubulin. Lower panel: summary of densitometric analysis expressed as a ratio by taking PMA stimulation alone as 1. C: COX-2 mRNA expression. Summary of analysis expressed as a ratio by taking PMA stimulation alone as 1. Bars show mean $\pm$ S.E. values ($n=3$ for B and D, 4 for C). vs. PMA under non-pressurized conditions. *** $p<0.001$ vs. PMA under non-pressurized conditions.

of pressure stress on COX-2 expression is dependent on the level of pressure because pressure stress of 80–120 mmHg had no effect on COX-2 expression. In detail, there are two possible reasons for the suppressive effect by high pressure; the one is the increase in the maximal pressure, *i.e.*, 160 vs. 120 mmHg. The another is the increase in the pressure gradient, *i.e.*, 80 (between 160 and 80) mmHg vs. 40 (between 120 and 80) mmHg. Further studies are required to clarify the detail condition of pressure loading to suppress the COX-2 expression. Also, we found that pressure stress alone had no effect on COX-2 expression. In contrast to our results, application of both shear and stretch within normal range to

endothelial cells has been reported to induce COX-2 protein expression without any stimulation.^{2,3,20)}

IL-1 β -induced COX-2 expression was inhibited by an ERK inhibitor, but not by a p38 MAPK inhibitor or a JNK inhibitor, suggesting that ERK activation was essential for the COX-2 expression in our experimental conditions. As mentioned in the Introduction, IL-1 β causes biphasic ERK activation, *i.e.*, the early stage and the late stage.^{9,10)} In the present study, we have clarified that pressure stress also inhibited the early stage of ERK phosphorylation, especially at 5 min after IL-1 β stimulation. On the other hand, ERK phosphorylation is reported to be augmented in the vasculature under excessive

Fig. 6. Suppressive Effect of the Pulsatile Atmospheric Pressure of 80–160 mmHg on PMA-Induced Transient ERK Activation in Rat VSMCs

A: The cells were either pretreated with or without 1 μ M SCH772984, 1 μ M SB 203580 or 1 μ M SP 600125 for 1 h, and then treated with 10 nM PMA for 1 h. B and C: The cells were treated with PMA for the indicated time periods (B) or 5 min (C) in the absence or the presence of the pressure stress of 80–160 mmHg. COX-2 protein expression and ERK activation were analyzed by Western blotting. COX-2 protein and phosphorylated ERK expression was normalized by α -tubulin and total ERK, respectively. A: Upper panel: representative blotting image of expressions of COX-2 and α -tubulin. Lower panel: Summary of densitometric analysis expressed by taking samples treated with PMA as 1. B: The representative protein expression of blotting images of COX-2 and α -tubulin, and phosphorylated and total ERK, respectively. C: Upper panel: representative blotting image of expressions of phosphorylated and total ERK. Lower panel: Summary of densitometric analysis expressed by taking samples treated with PMA under non-pressurized conditions as 1. Bars show mean $\pm$ S.E. values ($n = 3$ for A, 4 for C). * $p < 0.05$ vs. PMA under non-pressurized conditions.

pressurized conditions including spontaneously hypertensive rats in the established stage of hypertension, which contributes a vicious circle worsen hypertensive condition through

the proliferation of VSMCs.²¹⁾ Although, we have no clear explanation this contradiction with our present results, it is possible that the upregulation of ERK phosphorylation may be secondary to the elevated blood pressure and consequence of pathological changes in the levels of humoral factors. Further studies are required to clarify the effect of the pressure stress on the proliferation and migration of IL-1 β -stimulated cells.

The PKC family can be divided into three subgroups, *i.e.*, conventional PKC, novel PKC and atypical PKC, based on their structural characteristics and cofactor requirements. In human gingival fibroblasts and human myometrial smooth muscle cells, atypical PKC has an essential role for IL-1 β -induced COX-2 expression.^{22,23)} On the other hand, in some cell types COX-2 induction is mediated by conventional PKC.^{24,25)} In the present study, we have tested the role of PKC on IL-1 β -induced COX-2 expression in 3 different conditions, *i.e.*, 1) in the presence of 10 μ M of bisindolylmaleimide I, which inhibits all of three PKC; 2) in the presence of 2 μ M of bisindolylmaleimide I, which inhibits conventional and novel PKC; 3) in the presence of 2 μ M Gö 6976, which inhibits conventional PKC. As a result, IL-1 β -induced COX-2 expression was inhibited by inhibitors of all the conditions tested. These results suggest that conventional PKC plays an important role as a mechanism of the COX-2 expression induced by IL-1 β . In the present study, we also found that pulsatile pressure between 80–160 mmHg, but not 80–120 mmHg, suppressed PMA-induced COX-2 expression. Pressure stress suppressed PMA-induced COX-2 mRNA expression at 30 min after stimulation. These results suggest that the effect of pressure stress on intracellular signal transduction related to COX-2 expression occurs within 30 min after stress loading. Activation of PKC has been reported to induce COX-2 expression through MAPK including ERK in many cell types including VSMCs.^{12,26)} In fact, all the MAPK inhibitors we tested significantly inhibited PMA-induced COX-2 expression, suggesting both p38 MAPK and JNK signaling pathways are also necessary to induce COX-2. It has also been reported that PMA-induced COX-2 expression was inhibited by an ERK inhibitor, a p38 MAPK inhibitor and a JNK inhibitor in rat cardiac myocytes.¹²⁾ The α -isoform of PKC has been shown to directly phosphorylate Raf-1, providing a link to the ERK.²⁷⁾ Although the precise roles and mechanisms of p38 MAPK and JNK on PMA-induced COX-2 expression were not to be investigated in this study, the inhibition of ERK phosphorylation at the very early stage by pulsatile pressure stress may be involved, at least in part, in the mechanism of the inhibitory effect of COX-2 expression.

COX-2 expression in VSMCs is implicated in several pathological conditions including hypertension. High plasma levels of IL-1 β in a patient with essential hypertension have been reported.²⁸⁾ At the site of local vascular injury, IL-1 β is actively produced and released from activated macrophages. As prostaglandin I₂ has a vasodilating action and inhibitory effects on platelet aggregation and adhesion, leukocyte adhesion, and VSMC proliferation/migration, the induction of COX-2 in VSMCs may function as a defensive mechanism, and its disturbance may contribute to the development of cardiovascular disorders. Thus, pressure stress may affect the outcome of vascular injury *via* its action on COX-2 induction in VSMCs. In fact, we previously reported that IL-1 β -induced COX-2 expression in VSMCs isolated from stroke-prone spontaneously

hypertensive rats at the very early stage of evolution of hypertension is lower than that in those isolated from age-matched Wistar Kyoto rats.¹⁸⁾ Since pressure stress inhibits ERK activation at the early stage, it will be interesting to investigate whether the suppression of COX-2 expression by pressure stress is also observed with other stimulators such as angiotensin II¹⁶⁾ and lysophosphatidylcholine¹⁷⁾ in a future study.

In conclusion, pressure stress that simulates systolic hypertension suppresses IL-1 β - and PMA-induced COX-2 expression. Inhibition of ERK phosphorylation at the early and late stages by pressure stress may be involved in the mechanism of COX-2 inhibition. Downregulation of COX-2 expression in VSMCs by abnormal pressure stress may further worsen local vascular injury associated with hypertension.

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Conflict of Interest The authors declare no conflict of interest.

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