Lipid-Soluble Smoke Particles Upregulate Vascular Smooth Muscle ETB Receptors via Activation of Mitogen-Activating Protein Kinases and NF-kappaB Pathways

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Cigarette smoke is a strong risk factor for cardiovascular disease. However, the underlying molecular mechanisms that lead to cigarette smoke–associated cardiovascular disease remain elusive. With functional and molecular methods, we demonstrate for the first time that lipid-soluble cigarette smoke particles (dimethylsulfoxide-soluble cigarette smoke particles; DSP) increased the expression of endothelin type B (ETB) receptors in arterial smooth muscle cells. The increased ETB receptors in arterial smooth muscle cells was documented as enhanced contractility (sensitive myograph technique), elevated levels of arterial smooth muscle cells was enhanced in arterial smooth muscle cells. The increased ETB receptors in arterial smooth muscle cells involves activation of mitogen-activating protein kinases (ERK1/2 and p38) and the downstream transcriptional factor–kappaB (NF-κB) phosphorylation within 3 h. Blocking ERK1/2, p38, or NF-κB activation by their specific inhibitors significantly attenuated the DSP-induced upregulation of ETB receptor–mediated contraction. In conclusion, upregulation of ETB receptor–mediated contraction and both ETB receptor mRNA and protein expressions (immunohistochemistry and Western blotting). Intracellular signaling was studied with Western blotting and phosphoELISA; this revealed that DSP induced extracellular-regulated protein kinases 1 and 2 (ERK1/2), p38, and nuclear factor–κB (NF-κB) phosphorylation within 3 h. Blocking ERK1/2, p38, or NF-κB activation by their specific inhibitors significantly attenuated the DSP-induced upregulation of ETB receptor–mediated contraction and both ETB receptor mRNA and protein expression. In addition, dexamethasone abolished the DSP-induced upregulation of ETB receptor–mediated contraction. In conclusion, upregulation of ETB receptors by DSP in arterial smooth muscle cells involves activation of mitogen-activating protein kinases (ERK1/2 and p38) and the downstream transcriptional factor NF-κB pathways.

Key Words: smoking; ETB receptor; MAPK; VSMC; vascular disease.

In a previous study, we found that the lipid-soluble particles from cigarette smoke dimethylsulfoxide-soluble cigarette smoke particles (DSP) reduce the vasodilator function of vascular endothelial cells (VECs) via decreasing in the nitric oxide (NO)–mediated and the endothelium-derived hyperpolarization factor–mediated vasodilations in man and rat (Zhang et al., 2006). Recently, we have observed that DSP, at a concentration equivalent to the plasma level seen in smokers, induced upregulation of thromboxane A2 (TP) receptors in vascular smooth muscle cells (VSMCs) via posttranscriptional mechanism, mainly through enhanced translation of TP receptors (Zhang et al., 2008). Removal of the endothelium did not affect TP receptor–mediated contraction (Zhang et al., 2008), suggesting that the TP receptors on the VECs were not involved in the DSP effects. Similarly, organ culture of rat mesenteric arteries induces enhanced endothelin type B (ETB) receptor–mediated contraction, and the enhanced contraction is unaffected by removal of the endothelium (Adner et al., 1998a). More importantly, upregulation of the ETB receptor expression on the VSMCs is seen in cardiovascular disease (Li et al., 2007; Iwasa et al., 1999; Stenman et al., 2002). During organ culture of venous segments, the phenomenon of the ETB receptor upregulation is also induced on the VSMCs of the vein with a strong contraction mediated by the ETB receptors (Adner et al., 1998b). However, the underlying molecular mechanisms that are responsible for the ETB receptor upregulation are still not fully understood. The present study was designed to test the novel hypothesis that DSP increases the expression of ETB receptors on the VSMCs through activation of intracellular mitogen-activating protein kinases (MAPKs) and transcription factor nuclear factor–κB (NF-κB) inflammatory signal transduction pathways.

In fresh arteries, the endothelin type A (ETA) receptors are located on VSMCs and mediate contraction, while the ETB receptors are present on the endothelium and mediate vasodilatation via the release of nitric oxide (NO) and prostacyclin (Kedzierski and Yanagisawa, 2001). In experimental cerebral ischemia, however, the contractile ETB receptor phenotype is...
expressed on VSMCs and mediates strong vasoconstriction in the ischemic cerebral artery (Stenman et al., 2002). Increased expression of ETB receptors in VSMCs is also found in atherosclerotic plaques and neointimas (Iwasa et al., 1999). Thus, the expression of ETB receptors in VSMCs is considered as an important event in the development of cardiovascular disease.

We have observed that VSMC ETB receptor upregulation can be induced by organ culture of arterial rings in serum-free medium (Adner et al., 1996). This ETB receptor upregulation involves specific MAPK (Uddman et al., 2003). The present study demonstrates for the first time that during the organ culture of the arterial rings in the presence of DSP results in further enhanced expression of contractile ETB receptors in VSMCs via activation of the MAPK extracellular-regulated protein kinases 1 and 2 (ERK1/2) and p38, as well as the downstream transcription factor NF-kB inflammatory signal transduction pathways, subsequently resulting in upregulation of ETB receptors. Understanding the underlying molecular mechanisms that are responsible for smoking-associated cardiovascular disease may provide novel options for the treatment.

METHODS

Extraction of DSP. Cigarettes (Marlboro, 0.8 mg nicotine per cigarette) were “smoked” by an aspirator, and the smoke was directed through a cotton wool filter. The retained smoke particles from three cigarettes in the filter were dissolved in either 1 ml dimethyl sulfoxide (DMSO) (DSP) (Zhang et al., 2006) or in 1 ml distilled water-soluble cigarette smoke particles (WSP) and were diluted to a standard nicotine content of 0.1 mg/ml; these stock solutions were kept at −20°C for all subsequent experiments.

Tissue preparation and organ culture procedure. Sprague-Dawley rats (body weight 300–350 g) were anesthetized with CO2 and exsanguinated. The solution was continuously aerated with 5% CO2 in O2, resulting in a pH of 7.4. The VSMC ring segments were mounted for continuous recording of isometric tension with the Chart software (AD Instruments, Castle Hill, Australia) (Adner et al., 2008). Specific primers for the rat ETB receptor (GenBank accession no. NM_017333) were designed as follows—ETB receptor: forward: 5′-GATACGAC-AACCTTGCGTCCA-3′ and reverse: 5′-GTCCACAGTAGGACACATGAG-3′.

Elongation factor-1 (EF-1, GenBank accession no. NM_175838) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, GenBank accession no. NM_017008) mRNAs were used as references. The EF-1 and GAPDH primers were designed as follows—EF-1: forward: 5′-GAAGAGCCATGTGTTGTT-GAA-3′ and reverse: 5′-TGAATGACCAACAGCAAGCTG-3′ and GAPDH: forward: 5′-GGCTTCCGTGTCTTACC-3′ and reverse: 5′-CCTGTGTCGATCCACAA-3′.

Immunohistochemistry. Following organ culture in the presence of DMSO or DSP (0.2 μl/ml), the arterial segments were fixed, frozen in Tissue-Tek (Sakura Finetek Europe B.V., Zoeterwoude, Netherlands), cut at 10-μm thickness, and mounted on SuperFrost Plus slides (Menzel GMBH & COKG, Braunschweig, Germany) (Zhang et al., 2008). Immunohistochemistry staining with a specific polyclonal antibody against rat ETB receptor (Alexis Biochemicals, Lausen, Switzerland) and a secondary antibody donkey anti-goat sheep IgG conjugated to fluorescein isothiocyanate (Alexis Biochemicals) was used to demonstrate ETB receptor proteins. The stained arterial segments were observed under a confocal microscope (Nikon, C1plus, Nikon Instruments, Melville, NY) and analyzed by Image J software (http://rsb.info.nih.gov/jij) (Vikman et al., 2006). The fluorescence intensity was measured on the smooth muscle cells. For each vessel, six randomly selected sections were assessed. In each section, the fluorescence intensity was measured at 20 preset areas, and the mean fluorescence intensity was obtained from six vessels.

Western blot. After organ culture, vessels were frozen in liquid nitrogen and homogenized in cell extract denaturing buffer (BioSource, Invitrogen, Carlsbad, CA) containing a phosphatase inhibitor cocktail and protease inhibitor cocktail (Sigma, St. Louis, MO). Protein concentration was measured with a Bio-Rad protein analysis kit and TECAN infinite M200. Proteins (40 μg) were loaded and separated on 4–15% Ready Gel Precast Gels (Bio-Rad, Life Science Research, Hercules, CA), followed by probing with the One-Step Western Kit (GenScript Corporation, Piscataway, NJ) according to the manual. Briefly, membranes were incubated with pretreat solution on a shaker for 5 min at room temperature and then with a primary antibody overnight at 4°C. After washing three times for 10 min, the membranes were developed with working solution (LumiSensor A from arterial segments and C from arterial segments and activated protein-1 to demonstrate ETB receptor proteins. The stained arterial segments were observed under a confocal microscope (Nikon, C1plus, Nikon Instruments, Melville, NY) and analyzed by Image J software (http://rsb.info.nih.gov/jij) (Vikman et al., 2006). The fluorescence intensity was measured on the smooth muscle cells. For each vessel, six randomly selected sections were assessed. In each section, the fluorescence intensity was measured at 20 preset areas, and the mean fluorescence intensity was obtained from six vessels.

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The antibodies for phospho-p44/42 MAP kinase (Thr202/Tyr204), phospho-ERK1/2 (PD98059 and U0126), p38 (SB203580 and SB239063) and NF-κB were from Cell Signaling Technology (Beverly, MA) and purchased from Abcam (Cambridge, UK). All antibodies were used at 1:1000, and the experiments were repeated three times.

PhosphoELISA. VSMCs were isolated at 4°C from arterial segments and homogenized. Phosphorylated ERK1/2 was measured using a PhosphoELISA assay kit (BioSource International, Inc., CA) following the manufacturers’ instructions (Luo et al., 2006).

Differences and drugs. Selective ETB receptor agonist S6c, inhibitors for ERK1/2 (PD98059 and U0126), p38 (SB203580 and SB239063) and NF-κB (wedelolactone and IMD-0354), nonselective NF-κB and activated protein-1
Data analysis. All data are expressed as mean values ± SEM. The responses of S6c are presented as a percentage of contraction to 60 mM K. The amount of receptor mRNA relative to the internal control is expressed as a percentage of the control group. Statistical analysis was performed using one-way ANOVA and either Dunnet’s post-test or two-tailed unpaired Student’s t-test with Welch’s correction. A p-value less than 0.05 was considered to be significant.

RESULTS

DSP Enhances ET<sub>B</sub> Receptor Upregulation

Fresh mesenteric artery ring segments showed a weak contractile response to S6c (<5% of K<sup>+</sup>-induced contraction). Organ culture of the ring segments for 24 or 48 h resulted in markedly enhanced contraction induced by S6c (selective agonist for ET<sub>B</sub> receptor) (Figs. 1A and 1C). The K<sup>+</sup>-induced contraction did not differ between the groups. There was a significant increase in the contractile response to S6c in arterial segments exposed to DSP (0.2 or 0.4 μl/ml) for 24 or 48 h (Figs. 1A–C) as compared to control DMSO (p < 0.05). This suggests that the organ culture initiates the process of ET<sub>B</sub> receptor upregulation, and DSP accelerates this process. A concentration of 0.4 μl/ml DSP (for 24 h, Fig. 1B) caused a smaller contractile response to S6c as compared with 0.2 μl/ml incubation, suggesting the possible toxic effect of the high concentration of DSP to the VSMCs. Organ culture in presence of similar concentrations of DMSO as contained in DSP did not affect the contractile response to S6c. Removal of the endothelium did not affect the DSP effects. IRL2500 (10<sup>-5</sup> mol/l), a specific ET<sub>B</sub> receptor antagonist, blocked the contractile response to S6c with a pA<sub>2</sub> value of 8.04, demonstrating that the contraction was mediated by ET<sub>B</sub> receptors. In contrast, the extracted water-soluble cigarette smoke particles (WSP) (0.2 μl/ml) or nicotine alone (0.02 μg/ml, equivalent concentration of nicotine as in DSP) had no effects on the ET<sub>B</sub> receptor-mediated contraction (control E<sub>max</sub> 41±8% vs. WSP...
\(E_{\text{max}} 48 \pm 11\%\) and nicotine \(E_{\text{max}} 42 \pm 11\%; n = 8 \) in each group, and \(p > 0.05\). In addition, \(\text{ET}_A\) receptor–mediated contraction was examined after \(\text{ET}_B\) receptors had been desensitized (Zhang et al., 2004). The results showed that DSP enhanced the \(\text{ET}_A\) receptor–mediated contraction with an increased \(E_{\text{max}}\) from 260 \(\pm\) 3\% to 548 \(\pm\) 7\% \((n = 8, p < 0.001)\). However, angiotensin II–induced contraction was not affected by DSP.

\(\text{ET}_B\) receptor mRNA expression was assessed using real-time PCR. Organ culture of vessel segments in the presence of DSP (0.2 \(\mu\)l/ml) for 24 h enhanced the expression of \(\text{ET}_B\) receptor mRNA in the VSMCs, compared with control \((p < 0.001)\) (Fig. 1D).

Immunohistochemistry using specific antibodies against \(\text{ET}_B\) receptors demonstrated that the \(\text{ET}_B\) receptor protein was expressed in the VSMCs (Fig. 2A), and this was increased after exposure of the segments to DSP (Figs. 2B and 2C), compared with DMSO (control, \(p < 0.001)\). The enhanced \(\text{ET}_B\) receptor protein expression by DSP in VSMCs was further demonstrated by Western blot (Figs. 4A and 4E).

**DSP-induced Phosphorylation of MAPK ERK1/2, p38, and NF-\(\kappa\)B**

To explore intracellular signal transduction invoked by the smoke particles, we studied phosphorylated ERK1/2 (pERK1/2), p38 (pp38), and NF-\(\kappa\)B (pNF-\(\kappa\)B p65) by Western blot analysis of extracts of arterial smooth muscle ring segments cultured with DMSO (0.2 \(\mu\)l/ml, control) or with DSP (0.2 \(\mu\)l/ml) for 0, 1, 3, 6, or 24 h. Organ culture with DMSO per se significantly activated pERK1/2, pp38, and pNF-\(\kappa\)B p65 proteins with slightly different patterns. pERK1/2 was activated early (1 h), remained high until 6 h, and decreased slightly at 24 h (Fig. 3A). pp38 and pNF-\(\kappa\)B p65 increased more slowly and reached their highest levels at 6–24 h (Fig. 3A). DSP further enhanced the phosphorylation of the previous three signal proteins (Fig. 3A): for pERK1/2 at 1–6 h (Fig. 3B), pp38 at 3–24 h (Fig. 3C), and pNF-\(\kappa\)B p65 at 1–3 h (Fig. 3D). The increased phosphorylation of ERK1/2 by DSP is further confirmed by quantitative phosphoELISA measurements (Fig. 4F).

Specific ERK1/2 inhibitors PD98059 and U0126 significantly suppressed ERK1/2 activation (Figs. 4A and 4B) but had no effect on p38 (Figs. 4A and 4C). The activity of p38 (Figs. 4A and 4C), but not ERK1/2 (Figs. 4A and 4B), was inhibited by incubation with the p38 inhibitors SB203580 and SB239063. For pNF-\(\kappa\)B p65, its specific inhibitors (IMD-0354 and wedelolactone) had a weak effect, and only IMD-354 showed significant inhibitory effects (Figs. 4A and 4D), although both IMD-0354 and wedelolactone totally inhibited the DSP-induced upregulation of ETB receptor protein (Figs. 4A and 4E). All the specific inhibitors for ERK1/2 (PD98059 and U0126),

**FIG. 2.** Immunohistochemistry demonstrates an increased protein expression of ETB receptors in the VSMCs. Arteries were cultured for 24 h in the presence of (A) DMSO and (B) DSP (0.2 \(\mu\)l/ml). The arrowhead points to the collagen band (A), and the arrow points to the positive staining of ETB receptor protein (B). L: lumen. The size bar corresponds to 50 \(\mu\)m. (C) Semiquantitation of the receptor protein expression. Each data point is derived from six experiments. ***\(p < 0.001\) versus control (DMSO).
p38 (SB203580 and SB239063), and NF-κB (IMD-0354 and wedelolactone) inhibited DSP-induced ETβ receptor protein expression (Fig. 4E), respectively, but surprisingly, they did not markedly reduce the phosphorylation of NF-κB p65 protein in the VSMCs (Fig. 4D).

PhosphoELISA quantitative analysis showed that in the presence of specific ERK1/2 inhibitors (PD98059 and U0126), the DSP-induced ERK1/2 phosphorylation (Fig. 4F) was reduced by more than 60% and 80%, respectively (Fig. 4G).

**Inhibition of Intracellular Signal Transduction Attenuates DSP-enhanced Upregulation of ETβ Receptors**

Following 24 h of co-incubation with DSP together with specific inhibitors for ERK1/2 (PD98059 and U0126, Figs. 5A–C), p38 (SB203580 and SB239063, Fig. 5D–F), or NF-κB (IMD-0354 and wedelolactone, Figs. 5G–I), respectively, resulted in markedly reduced DSP-enhanced contractile responses to S6c. The $E_{\max}$ of contraction was depressed by inhibition of ERK1/2 (Fig. 5C), p38 (Fig. 5F), or NF-κB (Fig. 5I). However, a c-Jun N-terminal protein kinase (JNK) inhibitor (SP600125) did not modify the DSP effects on S6c responses (DSP plus SP600125, $E_{\max} = 175 \pm 26\%$ vs. DSP plus vehicle, $E_{\max} = 199 \pm 17\%$, $n = 8$, $p > 0.05$). Dexamethasone, an anti-inflammatory agent, abolished the DSP-enhanced contractile responses to S6c (Fig. 5J).

In parallel, we examined ETβ receptor mRNA and protein expression in the VSMCs as measured by real-time PCR and Western blot analysis, respectively. We observed that the initial increases in ETβ mRNA expression (Fig. 1D) and in protein expression (Fig. 2B) were abolished by the inhibitors for
FIG. 4. Examination of the specificity of inhibitors for ERK1/2 (PD98059 and U0126), p38 (SB203580 and SB239063), and NF-κB (IMD-0354 and wedelolactone), which were used in organ culture of the arterial smooth muscle rings in the presence of DSP for 6 h. (A) The pERK1/2, pp38 MAPK, and pNF-κB p65 proteins were determined by Western blot. The specificity of inhibitors for (B) ERK1/2 (PD98059 and U0126) and (C) p38 (SB203580 and SB239063) was confirmed. (D) Effects of all the inhibitors on pNF-κB p65 expression. (E) All tested inhibitors significantly abolished the DSP-induced increase in ET\textsubscript{a} protein expression. (F) PhosphoELISA experiments reconfirm that DSP (0.2 μl/ml) caused elevated pERK1/2 protein expression at 3 h, and (G) PD98059 and U0126 significantly inhibited the DSP-induced pERK1/2 protein expression, respectively. Data are shown as mean ± SEM. n = 6. *p < 0.05 and **p < 0.01 versus control.
FIG. 5. Contractile response of isolated mesenteric smooth muscle rings responded to S6c in a concentration-dependent manner. Inhibitors for ERK1/2 (A–C), p38 (D–F), and NF-κB (G–I) significantly attenuated the DSP-induced upregulation of ETα receptor-mediated contraction in the mesenteric arterial segments. The ERK1/2 (U0126), p38 (SB203580), and NK-κB inhibitors (IMD-0354) not only abolished the DSP effect but also blunted the ETα upregulation induced by organ culture per se. (J) The anti-inflammatory agent dexamethasone, which acts as a nonselective NF-κB and AP-1 inhibitor, abolished ETα-induced contraction. (K and L) ETα receptor mRNA expression in the VSMCs after organ culture with DSP (0.2 μl/ml) for 24 h in the presence of vehicle or inhibitors for ERK1/2, p38, and transcription (see above for explanation). The inhibitors DSP and DMSO were added to organ culture simultaneously at the beginning of the organ culture process. Values are shown as mean ± SEM, n = 7–8, **p < 0.01.
ERK1/2, p38, and transcription factor NF-κB (Figs. 5K, L and 4E), respectively. The reduction of ET_B receptor-mediated contraction paralleled the reduced expression of the ET_B receptor mRNA (Figs. 5K and 5L) and protein (Fig. 4E).

**DISCUSSION**

Cigarette smoke is a strong risk factor for cardiovascular disease. Studies have demonstrated that cigarette smoking acutely increases aortic stiffness and blood pressure in the smokers and the effects persist longer in smokers with hypertension than in the smokers without hypertension (Rhee et al., 2007). The stiffness of arteries is more prominent in chronic smokers (Kim et al., 2005). Cigarette smoking also causes immediate constriction of the coronary arteries with an increase in coronary resistance (Quillen et al., 1993). These acute hemodynamic effects may contribute to the adverse cardiovascular consequences of cigarette smoking. In stroke patients, cigarette smoking significantly increases the risk of acute attacks, and the risk is further increased in passive smokers (Bonita et al., 1999). A population-based cohort study in middle-aged adults concluded that both active and passive smoking are associated with the progression of atherosclerosis and this is of particular concern for patients with diabetes and hypertension (Howard et al., 1998). However, the underlying molecular mechanisms that lead to cigarette smoke-associated cardiovascular disease are poorly understood. The present study demonstrated that DSP can enhance ET_B receptor upregulation (at mRNA, protein, and functional levels) in VSMCs via activation of intracellular MAPK (ERK1/2 and p38) and the downstream transcriptional factor NF-κB (Figs. 5K, L and 4E), respectively. The reduction of ET_B receptor–mediated responses of cutaneous microcirculation (Edvinsson et al., 2008). In coculture models of the VSMCs with the endothelial cells, DSP via the VEC promotes the VSMC proliferation (Xu et al., 1991). In order to specifically address one aspect of the complicated interplay between the toxic effects of DSP and the arterial cells, the present study was designed to focus on the VSMCs. The functional alterations associated with the enhanced expression of ET_B receptors and dysfunction of endothelium by DSP may induce abnormal VSMC tone (via increased contraction and/or reduced relaxation) and enhanced VSMC proliferation. These functional alterations result in structure changes in the vascular wall associated with adverse remodeling, plaque formation, and in clinical sequelae such as myocardial infarction, stroke, and ischemia.

Our studies suggest that the intracellular MAPK and NF-κB inflammatory signal transduction pathways are involved in the upregulation of ET_B receptors in the VSMCs. In experimental rat subarachnoid haemorrhage (SAH), intracranial administration of specific inhibitors for MAPK ERK1/2 attenuates the expression of ETB receptors and prevents blood flow reduction after SAH (Ansar et al., 2007). In the present study, Western blot and PhosphoELISA use antibodies against specific phosphorylated (activated) key signal transduction molecules in order to elucidate the intracellular events activated by DSP. In a series of tests with a set of specific inhibitors for ERK1/2 (PD98059) (Alessi et al., 1995) and U0126 (Duncia et al., 1998), p38 (SB203580) (Baldassare et al., 1999) and SB239063 (Underwood et al., 2000), JNK (SP600125) (Bennett et al., 2001) and transcription factor NF-κB (IMD-0354) (Tanaka et al., 2005) and wedelolactone (Kobori et al., 2004), we obtained data that link the intracellular events with the upregulation of ET_B receptors in the VSMCs under DSP stimulation. We revealed that ERK1/2, p38, and NF-κB inhibitors significantly attenuated the additional DSP-induced upregulation of ET_B receptors, while the JNK inhibitor had no effect. The specific inhibitors for ERK1/2, p38, or NF-κB inhibited the DSP-induced ET_B receptor protein expression, suggesting that intracellular MAPK ERK1/2, p38, and NF-κB signal transduction pathways are involved. Surprisingly, the inhibitors did not markedly reduce the NF-κB p65 protein activity (phosphorylation) in the VSMCs. This suggests that their mode of inhibiting ET_B receptor expression were not through the NF-κB p65 protein phosphorylation or these inhibitors might directly prohibit the translocation of pNF-κB p65 into the cell nucleus, the key step for the downstream signaling of ERK1/2 and p38 MAPK (Hoffmann et al., 2006). The inhibitors used in the present study for blocking NF-κB signal transduction pathways have been demonstrated to prevent the phosphorylation and degradation of IκB (Tanaka et al., 2005; Kobori et al., 2004) and therefore they may block NF-κB translocation to the nucleus. Although all the ERK1/2, p38, and NF-κB inhibitors used in the present study significantly attenuated the enhanced ET_B receptor expression on the VSMCs, respectively, their effects may occur through different intracellular signal pathways. Furthermore, they may directly inhibit the process of ET_B receptor upregulation or indirectly block this process via up- and downregulation of other signal molecules like NO and AP-1. Interestingly, the
anti-inflammatory medication dexamethasone that is known to nonselectively inhibit NF-κB and AP-1 activity (Jeon et al., 2000) strongly diminished the DSP-induced upregulation of ET_B receptors. Taken all together, we have demonstrated that the activation of intracellular MAPK ERK1/2 and NF-κB inflammatory signal pathways is mainly responsible for the DSP-induced upregulation of ET_B receptors. However, we have recently determined that DSP induces thromboxane A_2 (TP) upregulation on the VSMCs with enhanced VSMC contraction. The upregulation of TP receptors by DSP occur via a posttranscriptional mechanism, mainly via enhanced translation of the receptor proteins and is independent of intracellular MAPK signaling (Zhang et al., 2008).

DSP contains lipid-soluble smoke particles that can pass through the lung alveolar membrane into the blood and are transported by low density lipoprotein to the arterial wall to participate in atherosclerotic plaque formation (Shu and Bymun, 1983). The WSP of cigarette smoke or nicotine per se did not induce changes in ET_B receptor expression in the VSMCs. This agrees with epidemiological studies that have demonstrated when tobacco is smoked, it leads to a high incidence of atherosclerotic cardiovascular disease, while using moist snuff or nicotine replacement does not have as many associated cardiovascular risks (Huhtasaari et al., 1992, 1999).

In conclusion, the present study has for the first time revealed that the expression of ET_B receptors in VSMCs is upregulated by DSP via activation of intracellular MAPK (ERK1/2, p38) and NF-κB inflammatory signal pathways. The upregulation of VSMC ET_B receptors enhances VSMC contraction and proliferation and thus may contribute to the development of cardiovascular disease. Understanding the underlying molecular mechanisms how the risk factors lead to cardiovascular disease may provide new options for the treatment.

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