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Up-regulation of Thromboxane A_2 receptor expression by lipid soluble smoking particles through post-transcriptional mechanisms

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Abstract

Atherosclerosis is a key factor in vascular disease and cigarette smoking is a well-known risk factor that may induce an inflammatory response and enhance plaque formation in arteries. Thromboxane (Tx) is one key inflammatory mediator involved in the pathogenesis of cardiovascular disease. The present study was designed to test if lipid soluble smoking particles (DSP) enhance TxA_2 receptor (TP) expression in rat mesenteric arteries, and if intracellular mitogen-activated protein kinase (MAPK) pathways play a role. Organ culture of rat mesenteric arteries in the presence of DSP (0.2 μl/ml for 24 hrs) resulted in markedly elevated contractile responses to the Tx analog U46619, compared with the control DMSO. There was no increase in TP receptor mRNA expression, while the protein expression was significantly enhanced. This up-regulation was not affected by a general transcriptional inhibitor actinomycin D, but was almost completely abolished by cycloheximide, a general translational inhibitor. Dexamethasone, a glucocorticoid, manifested a potent inhibitory effect as well. These results suggest that the up-regulation of TP receptor occurs via post-transcriptional events, and mainly translation. This is supported by experiments with specific inhibitors for c-Jun-NH_2-terminal kinase (SP600125), extracellular signal-regulated kinase 1 and 2 (PD98059 and U0126) and p38 (SB203580) that had no inhibitory effect on the up-regulation of TP receptors. Collectively, the results show that MAPK pathways are not involved in TP receptor up-regulation. Study on TP receptor mRNA stability showed that during organ culture, the TP receptor mRNA was stable in both DMSO and DSP group, but the latter elicited a tendency to stabilize the TP receptor mRNA at higher level. Thus, post-transcriptional mechanisms are responsible for the up-regulation of TP receptor by DSP, in which enhanced translation is the major cause of the elevated protein expression and the enhanced contraction.

Key Words: smoking; thromboxane; receptor; post-transcription; atherosclerosis; mesenteric; artery; rat.

Introduction

Cigarette smoking and second hand smoking (SHS), well-known risk factors, are associated with atherosclerosis (AS), coronary heart disease (CHD), stroke, myocardial infarction, aortic aneurysm, peripheral vascular disease and other cardiovascular diseases [1]. Smoking per se has been reported to result in 5 million premature deaths per year worldwide, quite interestingly the majority of these are due to a cardiovascular event [2]. It is considered that smoking particles cause damage to the arterial wall with localized dysfunction of the endothelium and enhanced plaque formation [3]. However, the molecular mechanisms involved in this phenomenon are still largely unknown.

Lipid soluble smoking particles (DSP) cause damage to vascular endothelial cells (VECs) and vascular smooth muscle cells (VSMCs) [4]; reduce the formation and release of prostacyclin from VEC [5, 6], increase ^{125}I-low density lipoprotein (LDL) uptake by VSMC; and together with LDL DSP increase the release of growth factors and endothelin-1 (ET-1) [7]. Furthermore, DSP may have a direct toxic effect on VEC and reduce endothelium-dependent dilatation in rat mesenteric arteries and human middle cerebral arteries [8]. As one putative mechanism, DSP result in up-regulation of endothelin A and B (ET_A and ET_B) receptors in rat bronchi via a translational mechanism [9].

Thromboxane (Tx) is widely known as a key inflammatory substance, and mediates VSMC contraction and proliferation via the thromboxane A_2 (TP) receptor [10, 11]. Tx is involved in blood platelet function and linked with hemostasis and thrombosis [12, 13]. The TP receptors density has been reported to be enhanced in cardiovascular disease [14] and in hypertension [15]. Previous experimental and clinical data have demonstrated that treatment with a TP receptor antagonist (e.g. Z-335, BM-573, Ramatroban (Bay U 3405), S18886)

provideds positive effects as shown both in vitro and in vivo [16-20]. Furthermore, injury-induced vascular proliferation and platelet activation are depressed in mice genetically deficient in the TP receptor or treated with a TP antagonist [21].

The present study was designed to examine if DSP up-regulates TP receptors in VSMC and molecular mechanisms bind this event. We show for the first time that DPS result in up-regulation of TP receptors in rat mesenteric artery SMC via a translational mechanism, while mitogen-activated protein kinase (MAPK) pathways are not involve.

Materials and Methods

Tissue preparation and organ culture procedure

Sprague-Dawley rats (body weight 300-350g) were anaesthetized with CO_2 and exsanguinated. The superior mesenteric artery was gently removed, immersed into cold buffer solution (for composition, see below) and freed of adhering tissue under a dissection microscope. The endothelium was denuded by perfusion of the vessel for 10 seconds with 0.1% Triton X-100 followed by another 10 seconds with a physiologic buffer solution [22]. The removal of the endothelium was verified by no dilatory response to acetylcholine (10^{-6}M) on 5-hydroxytriptamine (10^{-5}M) pre-contracted segments, and confirmed by immunohistology. The vessels were then cut into 1 mm long cylindrical segments, incubated at 37 °C in humified 5 % CO_2 in air. Culture was carried out in a 24-well plate, two segments in each well, containing 1 ml Dulbecco's modified Eagle's medium (DMEM) containing L-glutamine (584 mg/L) and supplemented with penicillin (100 U/mL) and streptomycin (100 µg/mL) [23]. The experiments were performed in organ culture of the arterial segments with DSP or dimethyl sulphoxide (DMSO). The experimental protocol was approved by Lund University Animal Ethic's Committee (M217-03).

Buffer solutions

Standard buffer solution (mM): NaCl 119; $NaHCO_3$ 15; KCl 4.6; MgCl 1.2; NaH_2PO_4 1.2; $CaCl_2$ 1.5; glucose 5.5. Analytical grade chemicals and double distilled water were used for preparing all solutions. DMEM, penicillin and streptomycin were purchased from Gibco BRL (Paisley, Scotland).

In vitro pharmacology

Arterial segments were immersed in temperature-controlled (37 °C) myographs (Organ Bath Model 700MO, J.P. Trading, Aarhus, Denmark) containing 5 ml bicarbonate buffer solution. The solution was continuously aerated with 5% CO_2 in O_2 resulting in a pH of 7.4. The arterial segments were mounted for continuous recording of isometric tension by the Chart software (ADInstruments, Hastings, UK). A resting tone of 2.5 mN was applied to each segment and the segments were allowed to stabilize at this tension for at least 1.5 hrs before being exposed to a potassium-rich (60 mM K^+) buffer solution with the same composition as the standard solution except that NaCl was replaced by an equimolar concentration of KCl. The potassium-induced contraction was used as a reference for the contractile capacity, and the segments were used only if potassium elicited reproducible responses over 1.0 mN. Concentration-response curves for vasoconstrictors were obtained by cumulative administration of the reagents [23].

Intracellular signal inhibitions and drugs

To link intracellular events to functional changes, a set of inhibitors were administered with DSP during organ culture. The MAPK pathway consists of three major members: c-Jun-

NH_2-terminal kinase (JNK), extracellular signal-regulated kinase 1 and 2 (ERK1/2) and p38 [24]. Their specific inhibitors including: SP600125 (10^{-5}M, inhibitor of JNK) [25], PD98059 and U0126 (10^{-5}M, inhibitors of ERK1/2) [26, 27] and SB203580 (10^{-5}M, inhibitor of p38) [28] were employed to examine the involvement of different MAPK subtypes. Actinomycin D (AcD, 5 mg/L) and cycloheximide (CHX, 10^{-5}M) were used as general transcriptional and translational inhibitors, respectively. The inhibitory effect of dexamethasone (DEX, 10^{-6}M), a potent anti-inflammatory glucocorticoid, was tested as well. The concentrations of these inhibitors were determined by recommendations from product information sheet and literatures.

All drugs were purchased from Sigma (St. Louis, USA). U46619 was diluted in ethanol to a stock concentration of 10 mM and diluted further in distilled water; the other reagents were dissolved in DMSO and stored according to the product information sheet and preparation guide.

Real-time reverse transcription (RT)-PCR

Smooth muscle cells were isolated from fresh or cultured artery segments and homogenized in 1 ml of the RNApro solution (Q-BIOgene, CA, USA) by using a FastPrep instrument (Q-BIOgene, CA, USA). The total RNA was extracted following a protocol from the FastRNA Pro kit supplier, and checked by GeneQuant Pro (Amersham Biosciences, Cambridge, UK) spectrophotometer. Reverse transcription of total RNA to cDNA was carried out using TaqMan Reverse Transcription Reagents (PE Applied Biosystems, CA, USA) in a Perkin-Elmer 2400 PCR (Perkin-Elmer, MA, USA) machine at 42 °C for 30 min. The real-time quantitative PCR was performed with the GeneAmp SYBR Green PCR kit in a GeneAmp 5700 sequence detection system (Perkin-Elmer, MA, USA). The system

automatically monitors the binding of a fluorescent dye to double-strand DNA by real-time detection of the fluorescence during each cycle of PCR amplification.

The PCR reaction was performed in a 50 μl volume and started at a temperature of 50 °C for 2 min, 95 °C for 10 min and the following 40 PCR cycles with 95 °C for 15 sec and 60 °C for 1 min. Dissociation curves were run after the real time PCR to identify the specific PCR products. All primers were designed using the Primer Express 2.0 software (PE Applied Biosystems, CA, USA) and synthesized by TAG Copenhagen A/S (Copenhagen, Denmark). Specific primers for the rat TP receptor mRNA (GenBank accession no. **NM_017054**) was designed as follows:

TP receptor	Forward: 5'- ATCTCCCATCTTGCCATAGTCC -3'
	Reverse: 5'- CCGATGATCCTTGAGCCTAAAG -3'

Elongation factor-1 (EF-1, GenBank accession no. **BC072542**) and glyceraldehyde 3-phosphate dehydrogenase (GADPH, GenBank accession no. **NM_023964**) mRNA were used as references, since they are the product of a housekeeping gene, continuously expressed to a constant amount in cells. The EF-1 and GAPDH primers were designed as follows:

EF-1	Forward: 5'- GCAAGCCCATGTGTGTTGAA -3'
	Reverse: 5'- TGATGACACCCACAGCAACTG -3'
GAPDH	Forward: 5'- GGCCTTCCGTGTTCCTACC -3'
	Reverse: 5'- CGGCATGTCAGATCCACAAC -3'

Data were analyzed with the comparative cycle threshold (C_T) method. To evaluate the amount mRNA in a sample, EF-1 and GAPDH mRNA were assessed in the same sample simultaneously. The C_T values of EF-1 and GAPDH mRNA were used as a reference to quantify the relative amount of TP receptor mRNA. The relative amount of mRNA was calculated with the C_T values of TP receptor mRNA in relation to the C_T values of EF-1 or GAPDH mRNA in the sample.

TP receptor mRNA stability

To study if a role of mRNA stability was involved in the up-regulation of TP receptors after organ culture with DSP, the segments were incubated with either DMSO or DSP (0.2 μl/ml) in the presence of AcD for ½, 3, 6 and 24 hrs, respectively. The administration of AcD resulted in inhibition of any de novo synthesis of receptor mRNA was inhibited. Real-time RT-PCR was used to determine the amount of TP receptor mRNA expression.

Immunohistochemistry

The arterial segments after organ culture in presence of DMSO or DSP (0.2 μl/ml) were immersed in a fixative solution consisting of 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) for 3 hrs at 4 ^{o}C. After fixation, the specimens were dehydrated in 20% sucrose of phosphate buffer (0.1M, pH 7.4) for 24 hrs at 4 °C, and then frozen in Tissue-Tek (Sakura Finetek Europe B.V., Zoeterwoude, Netherlands) and stored at -80 °C. Sections were cut at 10 μm thickness in a cryostat and mounted on SupperFrost Plus slides. Immunohistology staining with primary antibody against rat TP receptors from rabbits (Alexis Biochemicals, Lausen, Switzerland) and secondary antibody goat-anti-rabits IgG conjugated to fluorescein isothiocynate (FITC) (Alexis Biochemicals, Lausen, Switzerland) was used to demonstrate TP

receptor proteins. Briefly, the sections were incubated with the primary antibody (1:150 dilution) overnight at 4 °C, thereafter the secondary antibody (1:200 dilution) was applied for 1 hr at room temperature in dark. In the control experiments, either the primary antibody or the secondary antibody were omitted. The stained arterial segments were observed under a confocal microscope (Nikon, C1plus, Nikon Instruments Inc. NY, USA) and analysed by Image J software (http://rsb.info.nih.gov/ij) [29]. The measurement was done on positive staining on the smooth muscle cells. For each vessel, 6 sections were studied and the values obtained were mean fluorescence in the areas selected (there were 6 preset areas per section), and analyzed blindly as to the treatment protocol.

Extraction of DSP

DSP were extracted as previously described [8]. Briefly, three cigarettes (Marlboro, 0.8 mg nicotine per cigarette) were "smoked" by a water aspirator and the smoke was directed through a cotton wool filter. The retained smoke particles in the filter were dissolved in 1 ml DMSO and finally diluted by DMSO to standard nicotine content of 0.1 mg/L [9].

Data analysis

All data are expressed as mean ± SEM. The amount of receptor mRNA are expressed as percentage of housekeeping gene mRNA, or percentage of control group. Unpaired Student's t-test was used to compare two sets of data and one-way analysis of variance (ANOVA) or two-way ANOVA with Bonferroni post-test for comparisons of more than two data sets. A *P* value less than 0.05 was considered to be significant.

Results

In vitro pharmacology

The viability and general contractility of the arteries during organ culture in presence and absence of DMSO and DSP were examined by their contractile response to 60 mM K^+. The contractile responses to K+ were not significantly changed among the groups (fresh 2.58 ± 0.19 mN vs. organ culture 2.60 ± 0.20 mN, n=8, P > 0.05, and organ culture in the presence of DMSO 2.54 ± 0.29 mN vs. organ culture in the presence of DSP 2.47 ± 0.16 mN, n=8, P > 0.05). Organ culture of rat mesenteric arterial segments for 24 hrs with DSP 0.1 μl/ml did not affect the contractile responses to U46619 compared to the control group (Fig 1A). However, a higher DSP concentration (0.2 μl/ml) resulted in markedly elevated contractions compared with the DMSO control group (Fig 1B).

Removal of the endothelium did not affect the contractile responses to U46619 in both DMSO and DSP groups (U46619 Emax: DMSO with endothelium 7.25 ± 0.63 mN vs. DMSO without endothelium 7.15 ± 0.48 mN , n = 8, $P > 0.05$; U46619 pEC_{50}: DSP with endothelium 8.34 ± 0.46 vs. DSP without endothelium 8.66 ± 0.60 , n = 8, $P > 0.05$), indicating that the endothelium did not modify the reactions. In addition, organ culture itself induced a reduction in sensitivity of the smooth muscle cells to the thromboxane agonist (U46619 pEC_{50}: fresh 7.74 ± 0.03 vs. organ culture 7.00 ± 0.05, n = 8, $P < 0.05$), while the maximal contractile response to U46619 remained the same (U46619 Emax: fresh 6.58 ± 0.40 mN vs. organ culture 6.66 ± 0.38 mN , n = 8, $P > 0.05$).

The contractile responses to U46619 were shifted towards right by 10^{-8}M of the selective TP receptor antagonist, GR32191b, suggesting that U46619-induced contraction is mediated by TP receptor (data not shown).

Nicotine is an important substance in DSP. After organ culture for 24 hrs of the segments, the presence of nicotine (0.02 μg/ml, equivalent amount contained in DSP 0.2 μl/ml) did not result in any altered TP receptor-mediated contractile responses (Fig 1C).

Receptor mRNA expression and mRNA stability

TP receptor mRNA expression in VSMC was detected by real-time RT-PCR. After co-culture with DSP 0.2 μl/ml for 24 hrs it was not increased (Fig 2A, $P > 0.05$). In mRNA stability tests AcD was added to both DMSO and DSP groups to inhibit de novo synthesis of receptor mRNA for different time points of organ culture. The mRNA appeared to be quite stable in both groups. In parallel with the non-inhibited situation, DSP tended to stabilize mRNA and resulted in a slightly higher mRNA expression after 6 hrs though not significant (Fig 2B, $P > 0.05$).

Immunohistochemistry

The TP receptor protein was visualized by the fluorescent immuohistology method and analysed by confocal microscopy with the Image J software. Collagen bands (elastic fibres) appeared as orange autofluorescence and the TP-receptor proteins were stained clearly in green colour between the collagen bands. There was only a weak TP receptor protein expression in the DMSO group, localized among the smooth muscle cells (Figure 3A). However, the TP receptor protein was strongly enhanced in the DSP treated group as shown by the bright green granules localized on the SMC (Figure 3B). Semi-quantitative measurement of TP receptor protein density showed that DSP significantly enhanced TP receptor protein expression by 2 fold ($n = 6$, $P < 0.001$), compared with the control (DMSO) group (Figure 3C).

Inhibitor studies

The general transcriptional inhibitor AcD did not affect the elevated TP receptor-mediated responses elicited by DSP. However, the translational inhibitor CHX almost completely abolished the DSP-induced up-regulation of TP receptors. DEX, a glucocorticoid which shows extensive anti-inflammation effects, attenuated the up-regulation of TP receptor significantly (Fig 4A). Inhibition experiments of several intracellular pathways were carried out by using a set of specific inhibitors of various MAPK subtypes: JNK (SP600125), ERK1/2 (PD98059 and U0126) and p38 (SB203580). However none of these presented a significant effect (Fig 4B). Thus, a post-transcriptional mechanism is involved in the DSP-induced up-regulation of TP receptor expression.

Discussion

It is known that Tx and TxA_2 receptors are involved in the atherosclerotic process; enhanced contraction and proliferation of various cell types involved in the plaque formation [30]. Smoking presents an elevated risk factor enhancing this process. The present study is the first to demonstrate that organ culture of rat mesenteric arteries in the presence of DSP results in markedly elevated contractile responses to the thromboxane analog U46619 (selective agonist for TP receptors). The up-regulation of TP receptors after co-culture with DSP was shown also at the receptor protein level and localized to the smooth muscle cells in the vessel wall. A general transcriptional inhibitor AcD or subtype specific MAPK inhibitors did not affect the up-regulation of TP receptor induced by DSP, while CHX, a general translational inhibitor abolished the up-regulation. This suggests that post-transcription events are involved in the TP receptor up-regulation.

Studies have demonstrated that organ culture of rat mesenteric artery induces up-regulation of endothelin B (ET_B) receptor expression in the SMC. This occurs through de novo transcription and translation of the receptors via activation of MAPK pathways [31-33]. A set of inhibitors, previously tested, were used in the present study to examine if MAPK pathways are involved in DSP-induced up-regulation of TP receptors. The results showed that, specific inhibitors of JNK (SP600125), ERK1/2 (PD98059 and U0126) and p38 (SB203580) had no inhibitory effect on the DSP-induced up-regulation of TP receptors. These results agree with the inefficiency of AcD and further prove that the up-regulation of TP receptors does not occur via MAPK activated transcription. In contrast, we found that organ culture per se induces a decrease in sensitivity of the VSMC in response to selective TP receptor agonist U46619 via a transcription mechanism rather than translation (unpublished data).

In order to examine if the observation that there was no change in TP receptor mRNA was due to an artifact, mRNA stability experiments were carried out. Under the condition that de novo synthesis of receptor mRNA was inhibited, the TP receptor mRNA expression was stable at different time points of the organ culture progress. Despite of the observed nonsignificant differences, DSP showed a tendency to stabilize TP receptor mRNA expression compared with DMSO group. However, the marked increase in receptor protein expression indicates that a translational mechanism is the major cause of TP receptor up-regulation. In agreement with the present study, we have previously reported that DSP enhances ET_A and ET_B receptor expression through a translational mechanism in rat bronchioles [9]. The up-regulation of TP receptors may also occur through inhibition of the receptor protein break down mechanisms. However, CHX is a well-known general translational inhibitor that acts on both the synthesis of TP receptor protein and degrading enzymes for the TP receptors. If the degrading enzymes are inhibited by CHX, it could lead to

more TP receptor proteins and enhanced contractile responses to U46619. The present study showed that CHX had an inhibitory effect on the up-regulation of TP receptors. This suggests that CHX specifically acts on the translation process of TP receptors rather than degrading enzymes for TP receptors.

Previous investigations have revealed that TxA_2 generation is increased in aortic segments of cholesterol-fed atherosclerotic rabbits [34]; and that in two transgenic murine models of atherogenesis the Tx biosynthesis is elevated [35]. In addition, it has been reported that in coronary artery smooth muscle cells, thrombin-induced proliferation is markedly enhanced by TxA_2 and this involves up-regulation of TP receptor mRNA [36]. Thus, there exist data supporting that the TP receptor is an important target in prevention and treatment of atherosclerosis. Antagonists for TP receptors have achieved positive effects in experimental and clinical trials [16-20], and antagonism or deletion of TP receptor expression retards atherogenesis in apolipoprotein E knockout mice [37]. In our study, we have described a novel method for studies of the up-regulation of TP receptor expression induced by smoking particles which also suggests a possible role for active or passive cigarette smoking to promote atherogenesis and vascular diseases.

As a member of glucocorticoids family DEX exhibits non-specific anti-inflammation effects through several mechanisms. Firstly, it suppresses gene expression at the transcriptional level via inhibition of transcription factors such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) [38, 39]; secondly, post-transcriptional mechanisms account for the effects of DEX by depressing protein expression, including destabilization and translational inhibition of mRNA [40, 41]. In the present study, the inefficiency of the transcriptional inhibitor AcD as well as the specific MAPK inhibitors indicate that

transcription does not account for the up-regulation of TP receptors. Therefore, it is suggested that DEX has effect via a post-transcriptional process rather than a transcriptional mechanism. Glucocorticoids have been used in the treatment of experimental atherosclerosis [42, 43] and restenosis after coronary artery angioplasty [44], showing some anti-atherogenic effects. In human aortic smooth muscle cells, DEX may attenuate intimal proliferation at the site of vascular injury during atherogenesis [45].

In the present setup, DSP was quantified and standardized with nicotine content as an internal quantitative control for the DSP. The DSP preparations were analyzed by gas chromatography and then diluted by DMSO to standard nicotine content (0.1 mg/ml) [8, 9]. It has been reported that in smokers, the plasma concentration of nicotine varies between 4 - 72 ng/ml, and that the average concentration is 33 ng/ml [46]. In the present study, we used DSP 0.2 µl/ml which contains 20 ng/ml of nicotine. The used concentration is therefore slightly below the average plasma concentration in smokers, and thus our findings are relevant to *in vivo* both for active and passive smoking.

Nicotine, one of the key substances in smoking particles, was also examined in the present study. Equivalent amounts of nicotine as occur in DSP did not affect the contractile responses to U46619 after organ culture, suggesting that in our model nicotine is unrelated to the up-regulation of TP receptors. This finding is in concert with a previous study in which nicotine did not contribute to the increased production of TxA_2 following cigarette smoking in man no matter if it was studied *in vitro* or *in vivo* [47]. In a smoke exposed guinea pig model, the second-phase response of the bronchoconstriction characterized by the release of TxA_2, prostaglandin D_2, and prostaglandin $F_{2\alpha}$, which act on TP receptors in airway smooth muscles, was shown to be evoked by non-nicotine smoking particulates [48].

As a complex, smoking particles consist of more than 4000 different substances. It is difficult to verify which substances are mainly responsible for the up-regulation of TP receptors. Despite that the involvement of nicotine has been excluded here, reports have demonstrated that polycyclic aromatic hydrocarbons (dimethylbenzanthracene and benzpyren) and oxidants like free radicals are contained in DSP. These particles may result in dysfunction of the arterial cells [7, 49, 50] in aspects to cell proliferation, release of vasoactive substances as well as alteration of vascular receptors.

In conclusion, the present study is the first to conclusively demonstrate that smoking particles provoke up-regulation of TP receptors, with increased receptor expression and enhanced contractility in VSMC. This up-regulation occurs through a translational mechanism. The elevated TP receptor expression may contribute to the development of atherosclerosis and cardiovascular diseases.

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Figure Captions

Fig 1: U46619-induced contraction of rat mesenteric ateries after organ culture for 24 hrs in the presence of DMSO / DSP: (A) 0.1 μl/ml and (B) 0.2 μl/ml. Data are shown as the mean ± SEM. Each data point is derived from 8 segments. (C) TP receptor-mediated contraction of rat mesenteric arteries after organ culture for 24 hrs in the presence of nicotine 0.02 μg/ml (equivalent to DSP 0.2 μl/ml). In contrast with DSP, nicotine didn't elevate the TP receptor-mediated contractile response. Data are shown as the mean ± SEM. Each data point is derived from 7-8 segments.

Fig 2: (A) TP receptor mRNA expression in rat mesenteric artery after organ culture with DMSO or DSP (0.2 μl/ml) for 24 hrs. Data are expressed as mean ± SEM. Unpaired student's t-test, $P > 0.05$ vs. control group. Each data point is derived from 6 experiments. (B) TP receptor mRNA stability after co-culture with DMSO or DSP (0.2 μl/ml) for ½, 3, 6 and 24 hrs in the presence of AcD (5 mg/L). Data are expressed as mean ± SEM. Unpaired student's t-test, $P > 0.05$ vs. ½ hr group. Each data point is derived from 5 experiments.

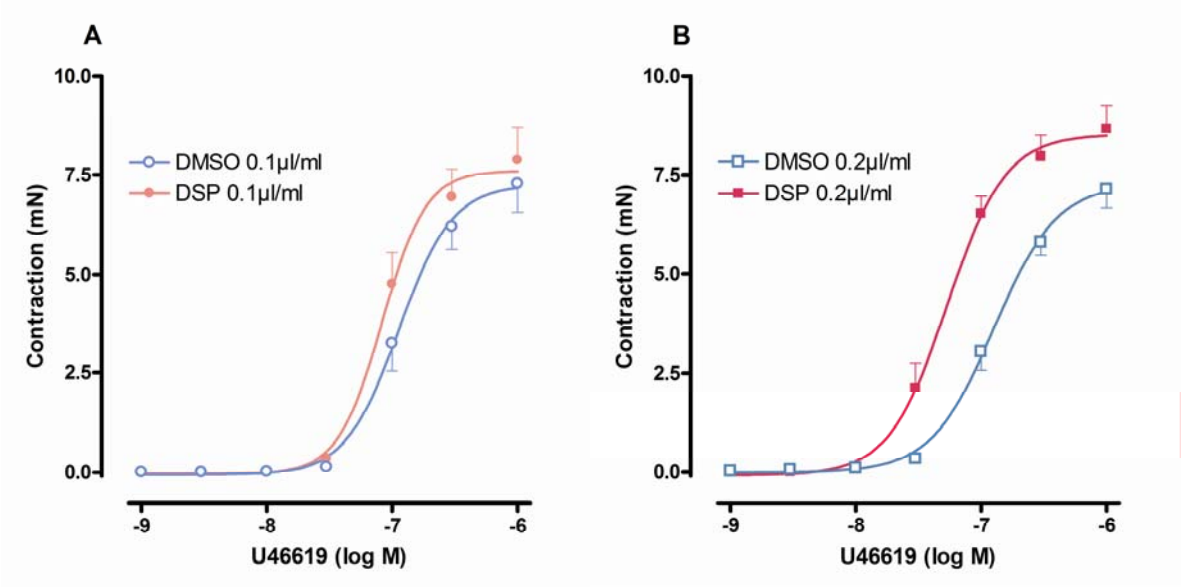
Fig 3: Immunohistology demonstrates an up-regulation of TP receptor protein expression. Arteries were cultured for 24 hrs in the presence of: (A) DMSO and (B) DSP (0.2 μl/ml). Arrows point to: (A) the collagen bands and (B) the TP receptor protein. The size bar corresponds to 10 μm. (C) semi-quantitation of the receptor protein expression. Each data point is derived from 6 experiments. Two-tailed unpaired Student's t-test with Welch´s correction, *** $P < 0.001$ compared with control (DMSO).

Fig 4: (A) DSP-induced up-regulation of TP receptor was not affected by a transcriptional inhibitor AcD. General translation inhibitor CHX almost completely abolished the up-

regulation. DEX, a glucocorticoid showed a potent inhibitory effect. Data are shown as the mean ± SEM. Each data point is derived from 6-12 segments. (B) Inhibition of JNK (SP600125), ERK1/2 (PD98059 and U0126) and p38 (SB203580) had no effect on DSP-induced up-regulation of TP receptor. Data are shown as the mean ± SEM. Each data point is derived from 7-10 segments.

Figures

Fig 1



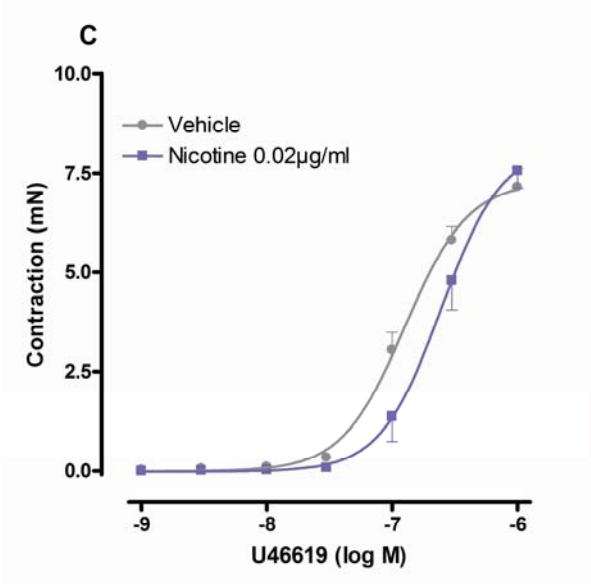


Fig 2

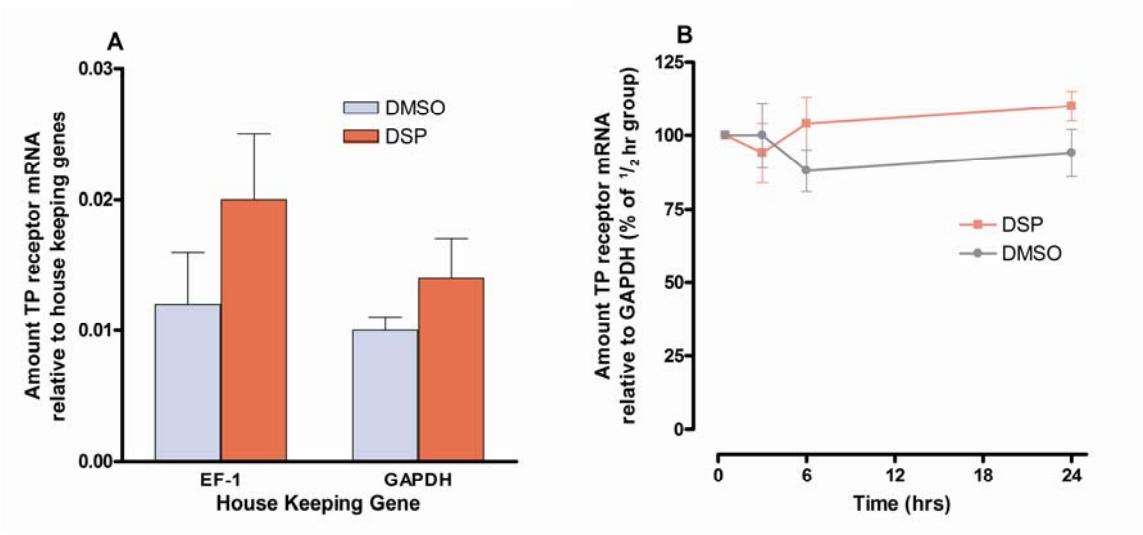
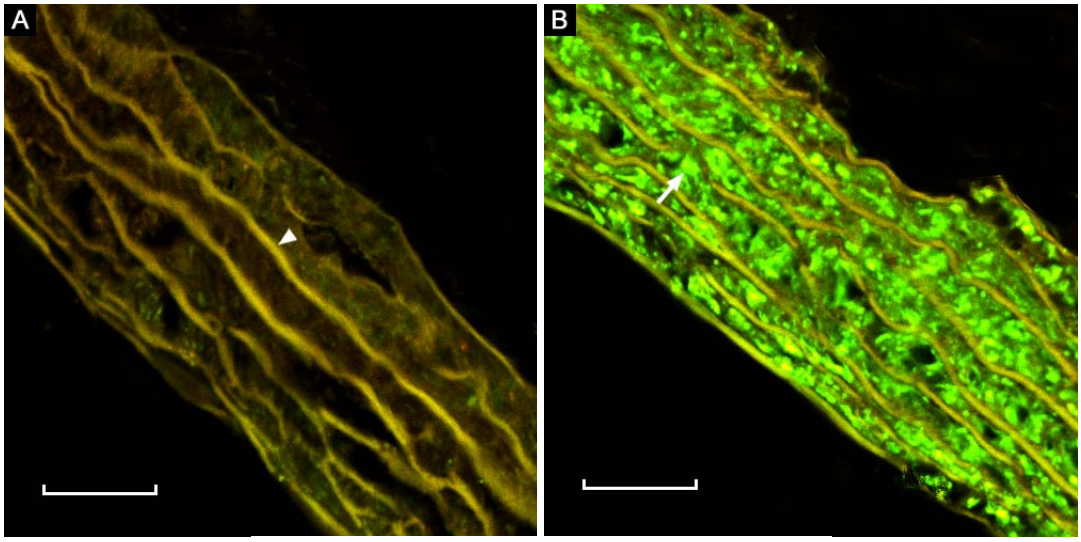

A
0.03
0.02
0.01
0.00
Amount TP receptor mRNA relative to house keeping genes
DMSO
DSP
EF-1
GAPDH
House Keeping Gene
B
125
100
75
50
25
0
Amount TP receptor mRNA relative to GAPDH (% of 1/2 hr group)
DSP
DMSO
0
6
12
18
24
Time (hrs)

Fig 3


A
B

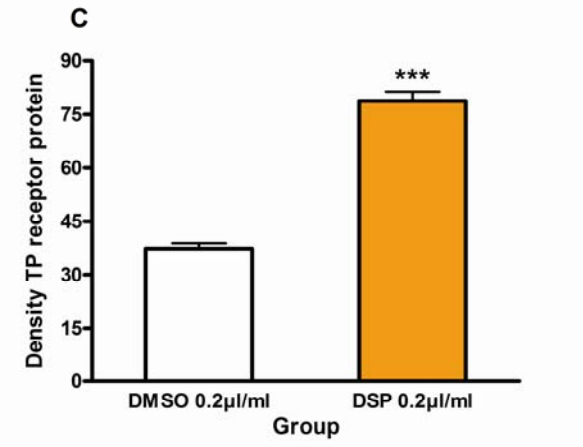
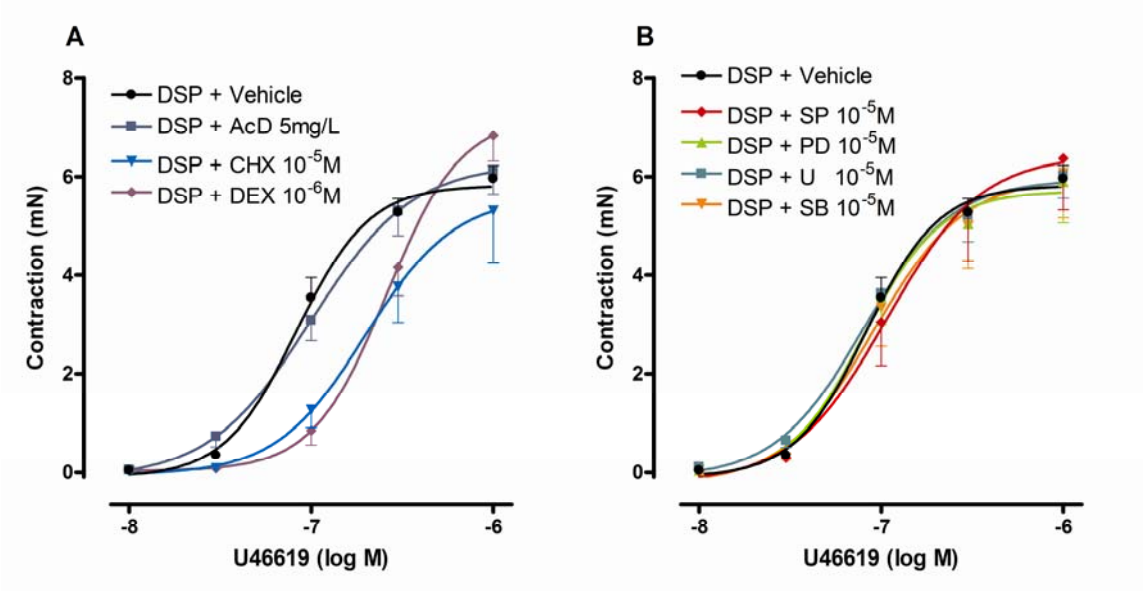

C
90
75
60
45
30
15
0
Density TP receptor protein
***
DMSO 0.2µl/ml
DSP 0.2µl/ml
Group

Fig 4


A
DSP + Vehicle
DSP + AcD 5mg/L
DSP + CHX 10-5M
DSP + DEX 10-6M
Contraction (mN)
U46619 (log M)
B
DSP + Vehicle
DSP + SP 10-5M
DSP + PD 10-5M
DSP + U 10-5M
DSP + SB 10-5M
Contraction (mN)
U46619 (log M)