Epigenetic Control of Microsomal Prostaglandin E Synthase-1 by HDAC Mediated Recruitment of p300

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Abstract (200 words)

Nonsteroidal anti-inflammatory drugs are the most widely used medicine to treat pain, inflammation and to inhibit platelet function. Understanding the expression regulation of enzymes of the prostanoid pathway is of great medical relevance. Histone acetylation crucially controls gene expression. We set out to identify the impact of histone deacetylases (HDACs) on the generation of prostanoids and examine the consequences on vascular function. Inhibition of HDACs (HDACi) with the pan-HDAC inhibitor SAHA attenuated prostaglandin E₂ (PGE₂) generation in the murine vasculature and in human vascular smooth muscle cells. In line with this, the expression of the key enzyme for PGE₂ synthesis, microsomal prostaglandin E synthase-1 (PTGES₁), was reduced by HDACi. Accordingly, the relaxation to arachidonic acid was decreased after ex vivo incubation of murine vessels with HDACi. To identify the underlying mechanism, chromatin-immunoprecipitation (ChIP) and ChIP-Seq analysis were performed. These results suggest that HDACs are involved in the recruitment of the transcriptional activator p300 to the PTGES₁ gene and that HDACi prevented this effect. In line with the acetyltransferase activity of p300, H3K27 acetylation was reduced after HDACi and resulted in the formation of heterochromatin in the PTGES₁ gene. Conclusion: HDAC activity maintains PTGES₁ expression by recruiting p300 to its gene.

Keywords: Epigenetics, prostaglandins, PGE₂, vascular biology, smooth muscle cells
Introduction

Prostanoids are important signaling molecules which are not only generated in the vascular system but also impact on several aspects of vascular biology. While some, such as prostacyclin (prostaglandin I2) are predominantly endothelium-derived, other like prostaglandin E2 are also produced by vascular smooth muscle cells. Basically all physiologically generated prostanoids alter vascular tone, gene expression and proliferative state. Vascular prostaglandin production is limited by the expression of the key enzymes of the prostaglandin pathway and by the availability of arachidonic acid. Altering enzyme expression, therefore, importantly impacts on vascular prostaglandin production. Several of these genes are subject to conventional transcription control, for example by inflammatory transcription factors like NFκB. It is, however, also becoming obvious, that large parts of gene expression are controlled by epigenetic mechanisms, which are also important in vascular gene expression control (1, 2).

Of these, histone modifications have a central role in controlling the chromatin structure. Particularly, histone acetylations at lysine residues have a strong impact on gene transcription. Acetylation transforms the condensed chromatin (heterochromatin) into a more relaxed structure (euchromatin) and thereby facilitates the access of the transcriptional machinery which results in increased gene transcription. The degree of acetylation on a lysine is dependent on the activity of histone acetyltransferases (HATs) and deacetylases (HDACs). Both enzyme classes have fundamental roles in biological processes, cell fate decisions, maintenance, survival and cancer development and thus are potential drug candidates (3, 4). Especially, HDAC inhibitors (HDACi) have been introduced in the clinic as novel anti-cancer drugs. Vorinostat (SAHA) was the first HDACi approved by the United States Food and Drug Administration and is used for the treatment of cutaneous T-cell lymphoma.

HDACi also affects the vascular system. In experimental models, HDACi reduces angiogenesis, restenosis and vascular inflammatory activity (4). HDACi also reduces endothelium-dependent relaxation as they reduce the expression of the endothelium nitric
oxide synthase (5). Besides nitric oxide (NO), arachidonic acid metabolites are the second most important vasoactive autacoids. However, little is known about the epigenetic regulation of the enzymes controlling vascular prostanoid production. For tumor cells, an unusual DNA methylation and histone acetylation has been reported by COX-1 and COX-2 in cancer (6–8). Whether HDACs impact on these enzymes in the vascular system is unknown and was studied here in the murine vasculature and human vascular smooth muscle cells.
Materials and Methods

Materials

SAHA, apicidin, prostaglandin E2 and diclofenac were purchased from Sigma-Aldrich. Arachidonic acid and U46619 were from Cayman Chemical. Deta NONOate was acquired from Enzo Life Sciences. Antibody against H3K9ac (#C15410004), H3K27ac (#pAb-174-050) and RNAP2 (C15100055) were from Diogenode. Antibody against p300 was from Bethyl (#A300-358).

siRNA transfection

For siRNA treatment, smooth muscle cells (80–90% confluent) were transfected with Lipofectamine 3000 according to the instructions provided by Thermo Fisher Scientific. siRNAs for HDACs, p300 and control siRNAs (siScrambled/siSCRs) were purchased from Thermo Fisher Scientific (Stealth RNAi), siHDAC1 # HSS104725, siHDAC2 # HSS104728, siHDAC3 # HSS113050, siHDAC4 # HSS114673, siHDAC7 # HSS147499, siHDAC8 # HSS125194, siEP300-1 #HSS103259, siEP300-2 #HSS103258 siSCR1 # 12935-300 and siSCR2 #12935112.

Cell Culture

Human vascular smooth muscle cells (human aortic smooth muscle cells (HAoSMC), #354-05a, human coronary artery smooth muscle cells (HCaSMC), # 350-05a and human carotid smooth muscle cells (HCtSMC) # 3514-05a were purchased from PELBiotech (Planegg, Germany). Cells were cultured on collagen type I coated- (#354236, Corning Incorporated, Tewksbury, MA USA) dishes in smooth muscle cell medium (#PB-MH-200-2190) supplemented with 8% fetal calf serum (FCS), penicillin (50 U/ml), streptomycin (50 μg/ml), EGF, FGF, glutamine and insulin from singlequots (PELOBiotech, Planegg, Germany). A humidified atmosphere of 5% CO2 at 37 °C was used. Cells were treated with SAHA 2 μmol/l or apicidin 200 nmol/l for 14 h in smooth muscle cell medium with 1% FCS.
Organ Chamber Experiments

Male C57/BL6 mice (10 weeks of age) were obtained from Charles River Laboratories, Sulzfeld, Germany. Isometric tension recordings were performed in an organ chamber setup with murine carotid rings (1-2 mm) in Krebs Henseleit buffer containing 2.2 g/l glucose at 37 °C and 5% CO₂. The concentration of the thromboxane receptor agonist U46619, used for preconstriction was adjusted to obtain an identical level of preconstriction (80%) relative to the contraction elicited by KCl (80 mmol/l). Arachidonic acid-induced relaxation was registered in the presence or absence of diclofenac 10 µmol/l.

Organ Culture

Murine carotid artery segments were dissected under sterile conditions, cleaned of adherent tissue, and incubated for 14 h under sterile conditions at 37°C in EBM culture medium containing 0.1% BSA, in the presence or absence of SAHA (2 µmol/l) in a conventional incubator at 5% CO₂. Subsequently, the tissue was used for organ chamber experiments and molecular biology.

Quantitative RT-PCR

Total RNA was extracted with the RNA Mini Kit (Bio&Sell). cDNA was prepared with Super-Script III reverse transcriptase (Invitrogen) and random hexamer together with oligo(dT) primers (Sigma #O4387). Quantitative real-time PCR was performed with Eva Green Master Mix and ROX as reference dye (Bio&Sell #76.580.5000) in a Mx3005 cycler (Stratagene). Relative expression of target genes was normalized to β-Actin and analyzed by the delta-delta Ct method with the MxPro software (Agilent Technologies, Santa Clara, CA, USA). The following two primer pairs for human PTGES1 were used: Forward (F1) TTG TCG CCT GGA TGC ACT TCC TGG; reverse (R1) AGG TGG CGG GCC GCT TCC CAG AGG, (F2) CGC TGC TGG TCA TCA AGA TGT ACG, (R2) TTT CCT GGG CTT CGT CTA CTC CTT and for murine Ptges1 (F) CAC TGC TGG TCA TCA AGA TGT ACG; (R) AAT GAG TAC ACG AAG
CGG AGG AAG. For p300 (F) TAG GAG TTC AAA CGC CGA GTC and (R) GTT GAG CTG
CTG TTG GCA TAG was used.

**Chromatin Immunoprecipitation (ChIP)**

Preparation of cell extracts, crosslinking and isolation of nuclei was performed with the
truCHIP™ Chromatin Shearing Kit (Covaris, USA) according to the manufacturers protocol.
The procedure was similar as described previously (9). After sonification of the lysates with the
Bioruptur Plus (10 cycles, 30 seconds on, 90 seconds off, 4°C; Diagenode, Seraing, Belgium),
cell debris was removed by centrifugation and the lysates were diluted 1:3 in dilution buffer (20
mmol/L Tris/HCl pH 7.4, 100 mmol/L NaCl, 2 mmol/L EDTA, 0.5% Triton X-100 and protease
inhibitors). Pre-clearing was done with 20 µL DiaMag protein A and protein G coated magnetic
beads slurry (Diagenode, Seraing, Belgium) for 45 min at 4°C. The samples were incubated
as indicated over night at 4°C with the antibodies indicated. 5% of the samples served as input.
The complexes were collected with 30 µL DiaMag protein A coated magnetic beads
(Diagenode, Seraing, Belgium) for 3h at 4°C, subsequently washed twice for 5 min with each
of the wash buffers 1-3 (Wash Buffer 1: 20 mmol/L Tris/HCl pH 7.4, 150 mmol/L NaCl, 0.1%
SDS, 2 mmol/L EDTA, 1% Triton X-100; Wash Buffer 2: 20 mmol/L Tris/HCl pH 7.4, 500
mmol/L NaCl, 2 mmol/L EDTA, 1% Triton X-100; Wash Buffer 3: 10 mmol/L Tris/HCl pH 7.4,
250 mmol/L lithium chloride, 1% Nonidet p-40, 1% sodium deoxycholate, 1 mmol/L EDTA) and
finally washed with TE-buffer pH 8.0. Elution of the beads was done with elution buffer (0.1 M
NaHCO₃, 1% SDS) containing 1x Proteinase K (Diagenode, Seraing, Belgium) and shaking at
600 rpm for 1h at 55°C, 1h at 62°C and 10 min at 95°C. After removal of the beads, the eluate
was purified with the QiaQuick PCR purification kit (Qiagen, Hilden, Germany) and subjected
to qPCR analysis. The following primers for quantification were used: -500 bp upstream of
PTGES1 TSS: (F) CAC AGT CAC GGG TTC TAG GGA TTG, (R) ACT CAG CCT GGA CAA
TGG AGC TGC; -1000 bp upstream of PTGES1 TSS: (F) GCA TTT GAC TGG GGA AAG AGT
TC, (R) GCT GTG TTG TTT TAA GCC ACT AAG; Exon 1 of PTGES1: (F) TGA TCA CAC
CCA CAG TTG AGC TGC, (R) ACA TAC CTT CTT CCG CAG CCT CAC; Exon 2 of PTGES1:
(F) ACT GGT ATA TTT CAG GCC TTT GC, (R) TGT GCA GAA GAA GTT CTG AAA GG.

Formaldehyde Assisted Isolation of Regulatory Elements (FAIRE)

FAIRE was performed similar as described (10, 11). 2x10⁶ VSMCs were crosslinked with 1% formaldehyde for 5 min and quenched with 125 mM glycine for 5 min. Afterwards, cells were scraped, washed with PBS and lysed in 2 mL FAIRE buffer 1 (50 mM HEPES-KOH [pH 7.5], 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton X-100) at 4°C for 10 min followed by centrifugation (5 min, 16,000 xg, 4°C). Cell pellets were resuspended and incubated in 2 mL FAIRE buffer 2 (10 mM Tris-HCl [pH 8.0], 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA) for 10 min at 22°C followed by centrifugation (5 min, 16,000 xg, 4°C). Cell pellets were resuspended in 400 µl FAIRE buffer 3 (10 mM Tris-HCl, [pH 8.0], 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% sodium deoxycholate, 0.5% N-lauroylsarcosine). Samples were sheared (30 sec On, 90 sec Off, 10 cycles, 4°C) with a Bioruptor Plus (Diagenode) to an average length of 200-400bp. Subsequently, samples were cleared by centrifugation (5 min, 16,000 xg, 4°C). 10% of each sample was used as input. Samples were further extracted with phenol/chloroform/isoamylalcohol (Roti Phenol/C/I) and aqueous phase was collected. Input and extracted DNA were de-crosslinked at 65°C for 6 h, followed by DNA purification with the QIAquick PCR purification kit (QIAGEN).

ChIP-Sequencing

Chip-Sequencing data of p300 was obtained from ENCODE, track name HeLa-S3 EP300 and visualized by the Integrative Genomics Viewer (IGV) Version 2.3.81 (127).

LC-MS/MS

Sample analysis was performed using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). The LC-MS/MS system consisted of a hybrid triple quadrupole-ion trap QTrap 5500 mass spectrometer (Sciex, Darmstadt, Germany) equipped with a Turbo-V-source operating in negative ESI mode, an Agilent 1200 binary HPLC pump...
and degasser (Agilent, Waldbronn, Germany) and an HTC Pal autosampler (Chromtech, Idstein, Germany). A cooling stack was used to store the samples at 4 °C in the autosampler. High purity nitrogen for the mass spectrometer was produced by a NGM 22-LC/MS nitrogen generator (cmc Instruments, Eschborn, Germany).

For the chromatographic separation a Synergi Hydro-RP column and precolumn were used (150 x 2 mm I.D., 4-µm particle size and 80 Å pore size from Phenomenex, Aschaffenburg, Germany). A linear gradient was employed at a flow rate of 300 µl/min. Mobile phase A was water/formic acid (100:0.0025, v/v, pH 4.0) and mobile phase B was acetonitrile/formic acid (100:0.0025, v/v). Sample solvent was acetonitrile/water/formic acid (20:80:0.0025, v/v, pH 4.0). Total run time was 16 minutes and injection volume of samples was 20 µl.

The mass spectrometer was operated in the negative ion mode with an electrospray voltage of -4500 V at 450°C. Multiple reaction monitoring (MRM) was used for quantification. The mass transitions used were m/z 351.1 → m/z 315.0 for PGE2 and PGD2, m/z 353.1 → m/z 309.2 for PGF2α, m/z 369.1 → m/z 163.0 for 6-keto-PGF1α, m/z 369.1 → m/z 169.1 for TXB2, m/z 355.1 → m/z 275.1 for [2H4]-PGE2 and [2H4]-PGD2, m/z 357.1 → m/z 313.2 for [2H4]-PGF2α, m/z 373.2 → m/z 167.1 for [2H4]-6-keto-PGF1α and m/z 373.1 → m/z 173.1 for [2H4]-TXB2 all with a dwell time of 50 ms (all the standards were purchased from Cayman Chemicals (Michigan, USA).

All quadrupoles were working at unit resolution. Quantitation was performed with Analyst Software V1.5 (Sciex, Darmstadt, Germany) using the internal standard method (isotope-dilution mass spectrometry). Ratios of analyte peak area and internal standard peak area (y-axis) were plotted against concentration (x-axis) and calibration curves for each prostaglandin were calculated by least square regression with 1/concentration^2 weighting.

**Statistics**

Unless otherwise indicated, data are given as means ± standard error of mean (SEM). Calculations were performed with Graphpad PRISM 5.0. For multiple group comparisons ANOVA followed by post hoc with Fisher LSD test was performed. In case of multiple testing,
Bonferroni correction was applied. Individual statistics of samples were performed by T-test. P values of <0.05 was considered as statistically significant. N indicates the number of individual experiments.
Results

Inhibition of HDACs reduces PGE\textsubscript{2} in the vasculature

To uncover the role of HDACs in the arachidonic acid metabolism, we determined the level of prostanoids in murine vessels incubated \textit{ex vivo} with the HDAC inhibitor SAHA (2µmol/l, 14h). Tandem mass spectrometry analysis revealed that the abundance of prostaglandin (PG) E\textsubscript{2} was markedly decreased, whereas PG\textsubscript{D2}, thromboxane (TX) B\textsubscript{2} and 6-keto-PGF\textsubscript{1a}, the marker for prostacyclin (PGI\textsubscript{2}), were not affected by HDACi (Fig. 1). Collectively, these findings suggest that HDAC activities are required for the PGE\textsubscript{2} levels in the vascular system.

\textit{PTGES1} expression is decreased by HDAC inhibition

The microsomal PGE\textsubscript{2} synthase (PTGES1) is the dominant enzyme responsible for the production of vascular PGE\textsubscript{2} from COX2-derived PGH\textsubscript{2} (12). Because PGE\textsubscript{2} levels were reduced by HDACi, we suspected that \textit{Ptges1} expression was also attenuated by SAHA, which was indeed the case (Fig. 2A). There was, in contrast, a trend towards a slight induction of \textit{Ptges2} und \textit{Ptges3}, which, however, did not reach the significance level. Moreover, these enzymes are not relevant for vascular prostaglandin formation. Smooth muscle cells are the predominant cell type in the vasculature. Therefore, we studied \textit{PTGES1} expression in combination with HDACi in human vascular smooth muscle cells (VSMCs). Consistent with the data obtained in the mouse vessels, human VSMCs exposed to 2 µmol/L SAHA expressed a significant lower level of \textit{PTGES1} as compared to control treated cells (Fig. 2B). Thus, our initial observation in murine carotid artery rings is also valid in human VSMCs. To determine whether reduction of \textit{PTGES1} was a result of reduced HDAC activity and not an unspecific effect of SAHA, a second inhibitor, apidicin was tested, which had a similar effect on \textit{PTGES1} expression (Fig. 2B). SAHA and apidicin are both canonical inhibitors, and thus reduce the activity of HDACs class 1 and 2 (13). To identify which HDAC is responsible for maintaining vascular \textit{PTGES1} expression, RNAi experiments were performed with two different control scrambled siRNAs. However, knockdown of several HDACs reduced \textit{PTGES1} (Fig. 2C),
suggesting that the effect of HDACi is a consequence of the concomitant inhibition of several HDACs like 1, 2, 4, 7 and 8.

**Inhibition of HDACs attenuates arachidonic acid-induced vessel relaxation**

To determine the relevance of HDAC-mediated PTGES1 induction, vascular reactivity assays were performed. Mice lacking Ptges1 exhibit an impaired conversion of arachidonic acid to the vasodilator PGE2, without changes in the level of other prostanoids (12, 14, 15). Therefore, we exposed preconstricted murine carotid rings to arachidonic acid as well PGE2 as positive control and analyzed their subsequent relaxation. The carotid vessels responded to PGE2 with relaxation (Fig. 3A). In line with the diminished Ptges1 expression after HDACi, the arachidonic acid-induced relaxation was attenuated by SAHA preincubation (Fig. 3B). Importantly, neither the relaxation to the NO donor DETA-NONOate nor the constriction to the thromboxane A2 receptor agonist U46619 was affected by HDACi (Fig. 3C&D). In order to demonstrate that an attenuated formation of vasodilator prostaglandins but not unrelated effects of arachidonate are responsible for the reduced relaxation after HDACi, experiments were performed in the presence of the cyclooxygenase inhibitor diclofenac. Importantly, diclofenac attenuated the relaxation to arachidonic acid in vessels of the control group but did not attenuate responses in vessels of the SAHA group. Thus, attenuated metabolism of arachidonic acid to a vasodilator compound is responsible for the HDACi effect (Fig. 3B). These data indicate that reduced HDAC activity in the vascular system attenuates arachidonic acid-induced relaxation, which could be attributed in part to decreased expression of PTGES1.

**Inhibition of HDAC abolishes p300 binding to the PTGES1 gene**

Histone acetylation is regulated by the competing activities of HDACs and histone acetyltransferases (HAT). The fact that PTGES1 expression was reduced by HDACi was unexpected. Since a direct action of HDACi should rather increase the acetylation of the PTGES1 gene and thus induce its expression. As this was obviously not the case for PTGES1, alternative modes of expression control were sought. For some genes HDACi decreases rather
than increases target acetylation (16, 17) but the mechanism underlying this effect is unclear. Potentially, HDACi attenuates HAT expression and thereby recruitment to its target genes (17). We therefore determined whether or not HDACi affects the recruitment of the transcriptional activators HATs. Chromatin immunoprecipitation (ChIP)-Seq data from ENCODE for a putative binding of HATs (GCN5, PCAF and p300) suggest that the PTGES1 gene is particularly occupied by the p300 HAT (Fig. 4A). Depletion of p300 by RNAi reduced PTGES1 expression in VSMCs, demonstrating that this HAT is indeed important for PTGES1 expression control (Fig 4B). Moreover, also in our hands, p300 binding to the PTGES1 gene was observed by ChIP (Fig. 4C). In accordance with our hypothesis of an attenuated HAT recruitment of HDACi, p300 abundance at the PTGES1 gene was largely attenuated after HDACi. (Fig. 4C). For these experiments the HDACi apicidin was used due to its higher potency. In line with a previous report (18), the reduced recruitment of p300 to the PTGES1 gene resulted in a reduced acetylation of the histone mark H3K27 but not H3K9 (Fig. 4D). Importantly, these effects were specific for the exon 1 of PTGES1 and not observed for the PTGES1 promoter: A strong enriched binding of p300 was not observed 500 and 1000 bp upstream of PTGES1 TSS and H3K27 at this region was rather induced (Fig. 4D).

HDACi not only attenuated acetylation of the PTGES1 exon 1 at H3K27, it also dramatically increased acetylation of H3K9 particularly in the promoter region (Fig. 4D). H3K9ac is an active mark and thus should result in PTGES1 expression. Thus, only direct determination of the chromatin state (i.e. hetero vs. euchromatin) can unravel the chromatin situation around the PTGES1 gene. For this purpose, a formaldehyde-assisted isolation of regulatory element (FAIRE) assay was carried out. In accordance with the reduced p300 binding and H3K27ac, more closed chromatin in the PTGES1 exon 1 and more open chromatin in the promoter region was observed (Fig. 4E). Finally, we addressed whether this closed chromatin environment could affect RNA polymerase 2 (RNAP2) occupancy. As recently, it was reported that HDACi can cause a block in the elongation step of transcription by pausing of RNA polymerase 2 (RNAP2) (19). In line with this hypothesis, we observed a dramatic accumulation of RNAP2 within the first exon of Ptges1 (Fig. 4F).
Collectively, these findings suggest that the enzymatic activities of HDACs are required for proper binding of the transcription activator p300 to the PTGES1 gene to facilitate H3K27ac and thereby euchromatin to enables proper RNAP2 elongation.

Discussion

In this study we determined the role of HDACs in some aspect of vascular arachidonic acid metabolism. Inhibition of HDACs reduced vascular PGE2 levels in the murine vasculature and in human vascular smooth muscle cells. This effect was a consequence of a reduced p300 recruitment to the Ptges1 gene.

PGE2 is a key product of arachidonic acid in the COX pathway. The lipid mediator has diverse functions including cytoprotection in the gastrointestinal tract, modulation of the immune system and induction of the febrile responses. Furthermore, in the vascular system, PGE2 reduced smooth muscle tone and changes proliferation and inflammatory activity (20–23). Here we observed that HDACi reduced vascular PGE2 level in the carotid which resulted in attenuated arachidonic acid-induced relaxation suggesting an attenuated conversion of arachidonic acid to the vasodilator PGE2 (12, 14, 15). The role of PGE2 in the control of the systemic blood pressure is under debate. Infusion of PGE2 into the kidney commonly causes renal vasodilation (20) and mice lacking PTGES1 exhibit an exaggerated hypertensive response to angiotensin II under normal conditions but not in a hyperlipidemic situation (20, 21, 23). In murine aortic rings, PGE2 directly induces relaxation through the E-prostanoid (EP) 4 receptor and thus cAMP and also data from our group suggest that the mouse carotid artery, a typical conduit vessel, relaxes to PGE2.

Acetylation of histone marks H3K27 and H3K9 results in euchromatin and facilitates access of transcription factors to genes. By removing these permissive marks, HDACs are mediators of gene repression. HDACi should therefore induce an open chromatin state. Thus, our observation that HDACi reduced PTGES1 expression in the murine vasculature and in VSMCs was unexpected. A simple explanation would be that HDACi not only induces, but also reduces
acetylation at certain sites. Indeed, recently two reports mediating HDACi with trichostatin A (TSA) and SAHA suggested that this can be the case in myocytes and endothelial cells (16, 17). Apparently, a similar mechanism is operative in the present study for PTGES1 in VSMCs.

Gene set enrichment analysis (GSEA) of RNA-Seq suggested that HATs could be influenced by HDACi. Particularly, reduced gene expression of p300-dependent genes were observed as a result of decreased p300 recruitment in TSA treated cells (17). Consistent with this, we observed that p300 occupancy to PTGES1 was also HDAC dependent. In fact, it was quite remarkable that acetylation of H3K27 a well-established target of p300 was strongly reduced by HDACi (24). In line with this, Jin et al. reported that deletion of GCN5/PCAF, a different HAT, specifically reduces acetylation on histone H3K9 while deletion of p300 specifically reduced acetylation on H3K27 (18). This suggests that due to the selective reduction in the recruitment of p300 the H3K27 but not the H3K9ac was decreased in response to HDACi. It is certainly unusual that such an epigenetic modification selectively occurs only in the PTGES1 gene especially in the first exon but not in the promoter. However, global run-on sequencing (GRO-seq) revealed that SAHA inhibit transcription of several genes by blocking RNAP2 elongation in the gene body (19). The analysis indicated that repressed genes by HDACi have an impediment within the gene locus. We hypothesize that such an impediment within the gene body could be achieved by changing the H3K27ac level and thus the chromatin environment into a tight state, which results in accumulation of RNAP2. Indeed, such mechanism e.g. is operating by co-transcriptional RNA splicing, histone acetylation is frequently used to accumulate RNAP2 and the spicing machinery over alternative exons (19, 25, 26)

In conclusion, HDACi results in decreased PTGES1 expression and vascular prostaglandin E2 formation as a consequence of attenuated recruitment of p300 to the PTGES1 gene.
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Disclosures

The authors declare that they have no relevant financial, personal or professional relationships to disclose which could be perceived as a conflict of interest or as potentially influencing or biasing the authors’ work.
References


Fig. 1. Inhibition of HDACs reduces PGE$_2$ in the vasculature. LC-MS/MS quantification of PGE$_2$ (A), PGD$_2$ (B), 6-keto-PGF$_{1α}$ (C) and TXB$_2$ (D) in mice carotid rings, incubated with SAHA (2 µmol/l, 14 h) or DMSO as control (Ctl). n = 5, *p < 0.05.
Fig. 2. PTGES1 expression is reduced by HDAC inhibiton (A&B) or depletion (C). A&B&C: qRT-PCR of the genes indicated (relative to β-actin) from murine carotid rings (A) and from human VSMCs (B&C). (A) Effect of SAHA (2 μmol/L, 14 h), (B) of SAHA or apicidin (14h) both relative to the control DMSO (Ctl). (C) Effect of two different control scrambled RNAi (siSCRs) or siRNA against HDAC as indicated. n ≥ 3, *p<0.05.
Fig. 3. Inhibition of HDACs reduces arachidonic acid-induced vessel relaxation. Vascular response assays of carotid rings. Relaxations to prostaglandin E2 (PGE$_2$, A) and arachidonic acid (100 nmol/l, B), in combination with and without diclofenac (diclo, 10 µmol/l) and the NO donor deta NONOate (C) in U46619 preconstricted vessels. (D) Contractions to the thromboxane receptor agonist U46619. Carotid rings were incubated with SAHA (2 µmol/l, 14 h) or with control solvent DMSO (Ctl). n = 7-10, *p < 0.05.
**Fig. 4.** Inhibition of HDAC abolishes p300 binding to the PTGES1 gene. (A) p300 ChIP-Seq of HEla cells from ENCODE. (B) qRT-PCR of PTGES1 (relative to β-Actin) from VSMCs with two different control scrambled siRNA (siSCRs) or two different siRNA against p300. (C&D&E) Chromatin immunoprecipitation and (E) formaldehyde-assisted isolation of regulatory elements (FAIRE) of VSMCs treated with control (ctl) solvent (DMSO) or apicidin (200 nmol/l, 14 h) with the antibodies against p300, H3K27ac, H3K9ac and RNAP2 (RNA polymerase 2) followed by qPCR for PTGES1 using primers binding at exon 1 (E1), exon2 (E2) or promoter region (500 or 1000 bp upstream of TSS). n = 3-6, *p < 0.05