ARTICLE

Extracellular PBEF/NAMPT/visfatin activates pro-inflammatory signalling in human vascular smooth muscle cells through nicotinamide phosphoribosyltransferase activity

**T. Romacho · V. Azcutia · M. Vázquez-Bella ·
N. Matesanz · E. Cercas · J. Nevado · R. Carraro ·
L. Rodríguez-Mañas · C. F. Sánchez-Ferrer · C. Peiró**

Abstract

Aims/hypothesis Extracellular pre-B cell colony-enhancing factor/nicotinamide phosphoribosyltransferase/visfatin (ePBEF/NAMPT/visfatin) is an adipocytokine, whose circulating levels are enhanced in metabolic disorders, such as diabetes mellitus and obesity. Here, we explored the ability of ePBEF/NAMPT/visfatin to promote vascular inflammation, as a condition closely related to atherothrombotic diseases. We specifically studied the ability of PBEF/NAMPT/visfatin to directly activate pathways leading to inducible nitric oxide synthase (iNOS) induction in cultured human aortic smooth muscle cells, as well as the mechanisms involved.

Methods iNOS levels and extracellular signal-regulated kinase (ERK) 1/2 activity were determined by western blotting. Nuclear factor (NF)-κB activity was assessed by electrophoretic mobility shift assay.

Results ePBEF/NAMPT/visfatin (10–250 ng/ml) induced iNOS in a concentration-dependent manner. At a submaximal concentration (100 ng/ml), ePBEF/NAMPT/visfatin time-dependently enhanced iNOS levels up to 18 h after stimulation. Over this time period, ePBEF/NAMPT/visfatin elicited a sustained activation of NF-κB and triggered a biphasic ERK 1/2 activation. By using the respective ERK 1/2 and NF-κB inhibitors, PD98059 and pyrrolidine dithiocarbamate, we established that iNOS induction by ePBEF/NAMPT/visfatin required the consecutive upstream activation of ERK 1/2 and NF-κB. The pro-inflammatory action of ePBEF/NAMPT/visfatin was not prevented by insulin receptor blockade. However, exogenous nicotinamide mononucleotide, the product of NAMPT activity, mimicked NF-κB activation and iNOS induction by ePBEF/NAMPT/visfatin, while the NAMPT inhibitor APO866 prevented the effects of ePBEF/NAMPT/visfatin on iNOS and NF-κB.

Conclusions/interpretation Through its intrinsic NAMPT activity, ePBEF/NAMPT/visfatin appears to be a direct contributor to vascular inflammation, a key feature of atherothrombotic diseases linked to metabolic disorders.

Keywords Adipocytokine · Atherosclerosis · Inducible nitric oxide synthase · Inflammation · PBEF/NAMPT/visfatin · Vascular smooth muscle

T. Romacho · V. Azcutia · M. Vázquez-Bella · N. Matesanz ·
E. Cercas · C. F. Sánchez-Ferrer · C. Peiró (✉)
Departamento de Farmacología y Terapéutica,
Facultad de Medicina, Universidad Autónoma de Madrid,
Arzobispo Morcillo, 4,
28029 Madrid, Spain
e-mail: concha.peiro@uam.es

J. Nevado
Instituto de Genética Médica y Molecular (INGEMM),
Hospital Universitario La Paz,
Madrid, Spain

R. Carraro
Servicio de Endocrinología, Hospital Universitario
de La Princesa y Universidad Autónoma de Madrid,
Madrid, Spain

L. Rodríguez-Mañas
Unidad de Investigación y Servicio de Geriatría,
Hospital Universitario de Getafe,
Getafe, Spain

Abbreviations

ePBEF/NAMPT/visfatin	Extracellular PBEF/NAMPT/visfatin
ERK 1/2	Extracellular-signal regulated kinase 1/2
HASMC	Human aortic smooth muscle cells
iNOS	Inducible nitric oxide synthase
NAMPT	Nicotinamide phosphoribosyltransferase
NF-κB	Nuclear factor-κB
NMN	Nicotinamide mononucleotide
PBEF	Pre-B cell colony-enhancing factor
PDTC	Pyrrolidine dithiocarbamate

Introduction

Extracellular pre-B cell colony-enhancing factor/nicotinamide phosphoribosyltransferase/visfatin (ePBEF/NAMPT/visfatin) has been identified as an adipocytokine preferentially released by visceral fat [1]. Whereas enhanced circulating ePBEF/NAMPT/visfatin levels have been reported in patients affected by metabolic disorders, such as diabetes mellitus, obesity or the metabolic syndrome [2, 3], the pathophysiological relevance of ePBEF/NAMPT/visfatin remains poorly understood. Enhanced circulating levels of ePBEF/NAMPT/visfatin have been positively associated not only with increased plasma levels of inflammatory markers [2], but also with vascular damage and endothelial dysfunction [4, 5]. An emerging hypothesis is that ePBEF/NAMPT/visfatin might directly promote vascular inflammation, therefore representing a link between metabolic disorders and atherothrombotic diseases exhibiting a chronic pro-inflammatory background, including atherosclerosis and CHD.

Despite this latter hypothesis, the ability of ePBEF/NAMPT/visfatin to exert direct pro-inflammatory effects on the vasculature remains largely unknown. To address this question, we used cultured human aortic smooth muscle cells (HASMC) to investigate the ability of ePBEF/NAMPT/visfatin to activate the extracellular-signal regulated kinase (ERK) 1/2–nuclear factor (NF)-κB–inducible nitric oxide synthase (iNOS) axis, whose activation is associated with vascular damage, inflammation and cardiovascular complications of diabetes [6]. We further aimed to identify the mechanism mediating the potential pro-inflammatory action of ePBEF/NAMPT/visfatin in human vascular smooth muscle cells.

Methods

Materials Culture plastic ware was from TPP (Tragadingen, Switzerland). DMEM, FCS and trypsin–EDTA were from Biological Industries (Beit-Hamek, Israel). Both human recombinant PBEF/NAMPT/visfatin and IL-1β were purchased from Peprotech (London, UK), with an endotoxin level <0.1 ng/μg. APO866 was kindly donated by Topotarget (Lausanne, Switzerland). The blocking mouse monoclonal antibody (Ab-3, clone 47-9) against the human insulin receptor (α-subunit) was purchased from Labvision (Fremont, CA, USA). Pyrrolidine dithiocarbamate (PDTC), ERK 1/2 inhibitor PD98059, human recombinant insulin, nicotinamide mononucleotide (NMN) and, unless otherwise stated, all other reagents were purchased from Sigma (St Louis, MO, USA).

Cell culture HASMC were obtained by enzymatic dissociation from the aortas of five organ donors, conforming with the principles outlined in the Declaration of Helsinki and accordingly to Spanish legal regulations [7]. HASMC were routinely cultured in DMEM supplemented with 10% (vol./vol.) FCS and antibiotics [7]. At confluence, HASMC were deprived of serum for 24 h prior to the addition of the different test compounds in serum-free medium supplemented with 0.1% (wt/vol.) BSA. Cultures between passages three and ten were used.

Western blotting Proteins (15 μg) from HASMC lysates were separated by SDS-PAGE, transferred to nitrocellulose membranes (Whatman, Maidstone, UK) and probed with a primary polyclonal antibody against iNOS (dilution 1/10,000; Transduction Laboratories, Lexington, KY, USA), followed by incubation with a horseradish peroxidase-conjugated secondary antibody (dilution 1/10,000; Chemicon, Temecula, CA, USA), as described previously [7]. Immunoreactive bands were detected by enhanced chemiluminescence (GE Healthcare, Uppsala, Sweden) and quantified using NIH ImageJ software (http://rsb.info.nih.gov/ij/, accessed 8 July 2005). The membranes were stripped and reprobed with an anti-α-tubulin primary antibody (Sigma; dilution 1/10,000) to ensure equal loading.

ERK 1/2 activity was determined by western blotting using specific antibodies against both the phosphorylated (activated) form of ERK 1/2 (dilution 1/1,000; Cell Signaling Technology, Danvers, MA, USA) and total ERK 1/2 (dilution 1/1,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA). ERK 1/2 activity was expressed as the phosphorylated ERK 1/2:total ERK 1/2 ratio.

Indirect immunofluorescence In HASMC, iNOS was visualised by indirect immunofluorescence, as previously described [7].

Nuclear extracts and electrophoretic mobility shift assay Nuclear extracts from HASMC cultures were prepared as

described before [7]. A commercial oligonucleotide (Promega, Madison, WI, USA) encoding the NF-κB consensus sequence (5′-AGTTGAGGGGACTTTC CCAGGC-3′) was 5′-end labelled using [γ-^{32}P]ATP and T4 polynucleotide kinase (Promega) and purified using MicroSpin G-25 columns (GE Healthcare, Chicago, IL, USA). For binding reactions, nuclear extracts (5 μg) were incubated on ice for 15 min in a reaction buffer (40 mmol/l HEPES [pH 7.0], 140 mmol/l NaCl, 5 mmol/l dithiothreitol, 10 μg/ml BSA, 0.01% [vol./vol.] Nonidet P-40, 4% [wt/vol.] Ficoll and 0.05 μg/ml poly [dI-dC]). After addition of the labelled oligonucleotide (~50,000 cpm) the reaction mix was further incubated for 20 min at room temperature. For competition experiments a 100-fold excess of unlabelled doubled-stranded oligonucleo-tides was added to the binding reaction. DNA–protein complexes were resolved on 4% (wt/vol.) non-denaturing polyacrylamide gels in 0.5× TBE (45 mmol/l TRIS–borate, 1 mmol/l EDTA, pH 8.0) at 4°C. Gels were dried and exposed to autoradiography at −80°C.

Statistical analysis Results are expressed as means±SEM of at least three independent experiments. Statistical analysis was performed using ANOVA for curves or Student's *t* test for data points, with the level of significance chosen at $p < 0.05$.

Results

ePBEF/NAMPT/visfatin induces iNOS in HASMC through an ERK 1/2- and NK-κB-dependent mechanism In HASMC exposed to ePBEF/NAMPT/visfatin (10–250 ng/ml) for 18 h, a concentration-dependent increase in the levels of the pro-inflammatory enzyme iNOS was observed, with a threshold concentration of 50 ng/ml (Fig. 1a). Figure 1b depicts the time course of iNOS induction by a sub-maximal concentration of ePBEF/NAMPT/visfatin (100 ng/ml): iNOS levels were significantly enhanced 8 h after the addition of the adipocytokine and peaked at 18 h, with no further induction of iNOS observed at 24 h (96.6±12.1% of the levels achieved at 18 h; results from three independent experiments). The pro-inflammatory adipo-cytokine IL-1β (10 ng/ml) was used as a well-known positive control of iNOS induction (3.3±0.3-fold over basal cultures; $p < 0.05$, results from five independent experiments, Fig. 1b). The induction of iNOS by ePBEF/ NAMPT/visfatin (250 ng/ml) or IL-1β (10 ng/ml) in HASMC was visualised by indirect immunofluorescence (Fig. 1c).

In vascular smooth muscle, the induction of iNOS by IL-1β is regulated through the activation of the transcription factor NF-κB, which in turn requires the upstream activation
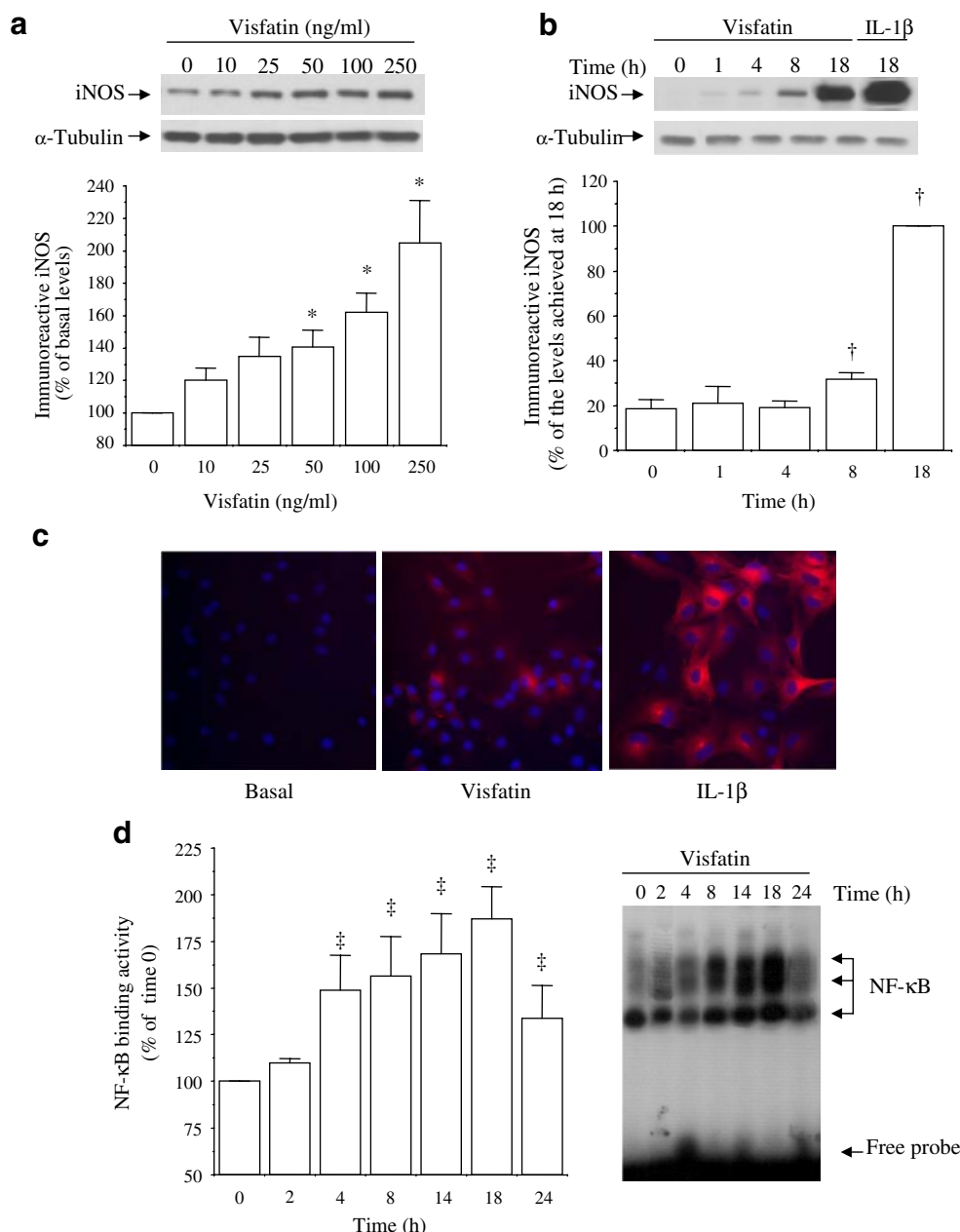
of the mitogen-activated protein kinase ERK 1/2 [8]. In HASMC, ePBEF/NAMPT/visfatin (100 ng/ml) elicited a gradual increase in NF-κB binding activity from 2 to 18 h, when the maximal activation was attained (Fig. 1d). At 24 h, NF-κB activity was about 50% of that achieved at 18 h, although it remained enhanced compared with basal levels (Fig. 1d). IL-1β yielded a 2.7±0.4-fold increase over basal NF-κB activity after 1 h of treatment ($p < 0.05$; results from three independent experiments). The addition of a 100-fold excess of cold probe blunted the formation of the binding complexes, demonstrating their specificity (data not shown).

In addition, ePBEF/NAMPT/visfatin (100 ng/ml) triggered a biphasic time-dependent activation of ERK 1/2 in HASMC: at times <1 h, a transient increase in the phosphorylated ERK 1/2:total ERK 1/2 ratio was observed at 10 min, which decayed to basal levels between 30 min and 1 h (Fig. 2a). Later on, a gradual and sustained activation of ERK 1/2 was observed up to 18 h (Fig. 2a). The activation of ERK 1/2 by ePBEF/ NAMPT/visfatin (10–250 ng/ml) was concentration-dependent, as shown in Fig. 2b. IL-1β elicited a 3.2± 0.7-fold increase over basal ERK 1/2 activity after 18 h of treatment ($p < 0.05$; results from three independent experiments).

We next assessed the implication of both NF-κB and ERK 1/2 in ePBEF/NAMPT/visfatin-mediated iNOS induction in HASMC. The co-incubation of ePBEF/ NAMPT/visfatin (100 ng/ml) with either the ERK 1/2 inhibitor PD98059 (30 μmol/l) or the NF-κB inhibitor PDTC (100 μmol/l) suppressed the induction of iNOS by the adipocytokine (Fig. 2c). Neither PD98059 nor PDTC alone modified basal iNOS levels (data not shown). Furthermore, PD98059 abolished NF-κB activation trig-gered by PBEF/NAMPT/visfatin (Fig. 2d), highlighting the role of ERK 1/2 in NF-κB activation by PBEF/ NAMPT/visfatin.

The pro-inflammatory action of ePBEF/NAMPT/visfatin in HASMC is not mediated by the insulin receptor To gain insight into the mechanisms mediating the pro-inflammatory action of ePBEF/NAMPT/visfatin in HASMC, we next explored the role of the insulin receptor, as ePBEF/NAMPT/visfatin was initially proposed as an insulin mimetic [1]. For this purpose, we used an antibody that binds the α-subunit of the insulin receptor and blocks the receptor activation [9, 10] and examined NF-κB activation as a key molecule regulating inflammation-related genes. HASMC pre-incubation for 2 h with the blocking antibody did not affect NF-κB activation by ePBEF/NAMPT/visfatin (100 ng/ml) after 18 h (Fig. 3), and did not modify the effect of IL-1β (10 ng/ml), used as a negative control (Fig. 3). Contrarily, the antibody

Fig. 1 ePBEF/NAMPT/visfatin stimulates iNOS induction and NF-κB binding activity in HASMC. a HASMC were challenged with increasing concentrations of visfatin (10–250 ng/ml) and 18 h later iNOS levels were determined by western blotting. *$p<0.05$ vs cultures without visfatin. A representative gel is shown at the top. b Time course of iNOS induction by visfatin (100 ng/ml). †$p<0.05$ vs time 0. A representative gel is shown at the top, in which the effect of IL-1β (10 ng/ml), used as a positive control for iNOS induction, is shown. c Immunolocalisation of iNOS by indirect immunofluorescence in HASMC cultures untreated (basal) or treated with visfatin (250 ng/ml) or IL-1β (10 ng/ml) for 18 h. Nuclei were counterstained with DAPI. Magnification ×400. d Time course of NF-κB activation by visfatin (100 ng/ml). ‡$p<0.05$ vs time 0. A representative gel is shown on the right. Means±SEM of at least three independent experiments

NAMPT enzymatic activity mediates the pro-inflammatory action of ePBEF/NAMPT/visfatin in HASMC As it has been reported that ePBEF/NAMPT/visfatin may exert its cellular actions through its intrinsic NAMPT enzymatic activity [11], we first explored whether NMN, the product of NAMPT activity and a precursor of NAD [12], could mimic the pro-inflammatory effect of PBEF/NAMPT/visfatin in HASMC. Figure 4a shows that exogenous NMN (100 μmol/l) stimulated NF-κB activity to a similar extent as ePBEF/NAMPT/visfatin (100 ng/ml). NMN (100 μmol/l) also triggered iNOS induction in HASMC, which was not significantly different from that achieved by 100 ng/ml ePBEF/NAMPT/visfatin (Fig. 4b).

We next assessed the impact of NAMPT activity inhibition on the pro-inflammatory action of ePBEF/NAMPT/visfatin. As shown in Fig. 4c, the activation of NF-κB elicited by ePBEF/NAMPT/visfatin (100 ng/ml) in HASMC was completely abolished by co-incubation with the NAMPT activity inhibitor APO866 (100 nmol/l) [13]. Analogously, APO866 (100 nmol/l) blocked iNOS induction by 100 ng/ml ePBEF/NAMPT/visfatin (Fig. 4d). APO866 itself did not modify NF-κB activity or iNOS
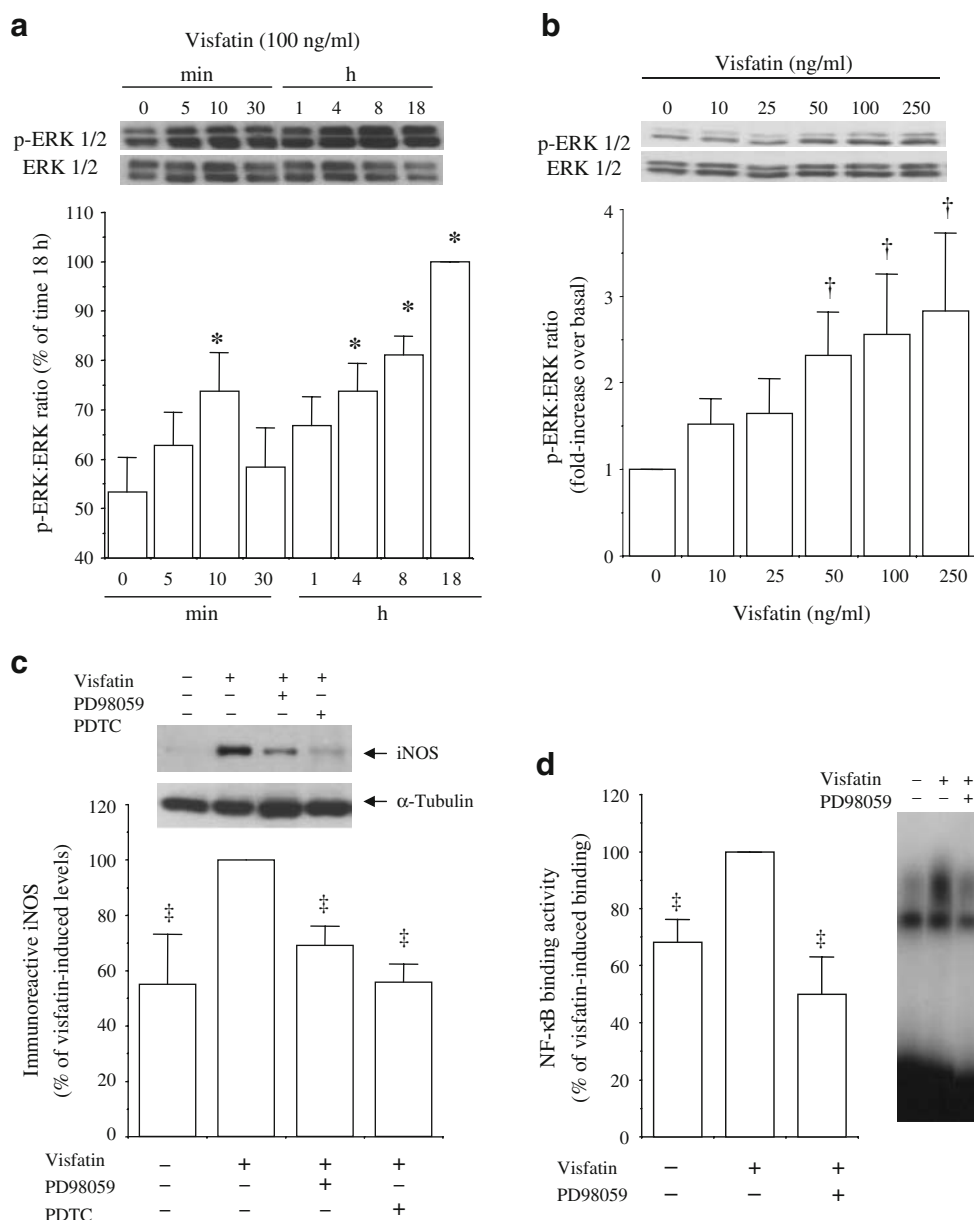
significantly reduced NF-κB activation by insulin (10 nmol/l), used as a positive control of the receptor activation (Fig. 3).

Fig. 2 iNOS induction by ePBEF/NAMPT/visfatin is dependent on ERK 1/2 and NF-κB sequential activation. **a** Time course of ERK 1/2 activation by visfatin (100 ng/ml). ERK 1/2 activity was expressed as the phosphorylated ERK (p-ERK) 1/2:total ERK 1/2 ratio. *$p<0.05$ vs time 0. A representative gel is shown at the top. **b** Concentration-dependent activation of ERK 1/2 after 18 h of stimulation with visfatin (10–250 ng/ml). †$p<0.05$ vs cultures without visfatin. A representative gel is shown at the top. **c** iNOS levels in HASMC exposed for 18 h to visfatin (100 ng/ml), alone or in the presence of the ERK 1/2 inhibitor PD98059 (30 μmol/l) or the NF-κB inhibitor PDTC (100 μmol/l). ‡$p<0.05$ vs visfatin alone. A representative immunoblot is shown at the top. **d** Visfatin-induced NF-κB binding activity is dependent on ERK 1/2 activity. NF-κB activity was determined in HASMC exposed for 18 h to visfatin (100 ng/ml) alone or in the presence of PD98059 (30 μmol/l). ‡$p<0.05$ vs visfatin alone. Means±SEM of at least three independent experiments

levels in non-stimulated HASMC (Fig. 4c,d). It is worth noting the NAMPT substrate nicotinamide is contained in the culture medium of HASMC (DMEM).

Discussion

In the present study, we have demonstrated the ability of the adipocytokine PBEF/NAMPT/visfatin to exert a direct activation of pro-inflammatory signals in human vascular smooth muscle. Indeed, ePBEF/NAMPT/visfatin upregulated the levels of iNOS, a nitric oxide- and peroxynitrite-forming enzyme that plays a key role in vascular inflammation and endothelial dysfunction, and which is overproduced in vascular diseases like atherosclerosis or diabetes-associated vasculopathy [6]. Analogously to the pro-inflammatory and pro-atherogenic adipocytokine IL-1β, the induction of iNOS by PBEF/NAMPT/visfatin required the sequential upstream activation of ERK 1/2 and NF-κB [8]. This latter transcription factor, which plays a pivotal role in the transcriptional regulation of multiple inflammation-related genes, is activated by ePBEF/NAMPT/visfatin in another vascular cell type, the endothelial cell, resulting in the production of intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and other mediators of inflammation [14, 15].

It is worth noting that the pro-inflammatory action of ePBEF/NAMPT/visfatin in HASMC was observed from a
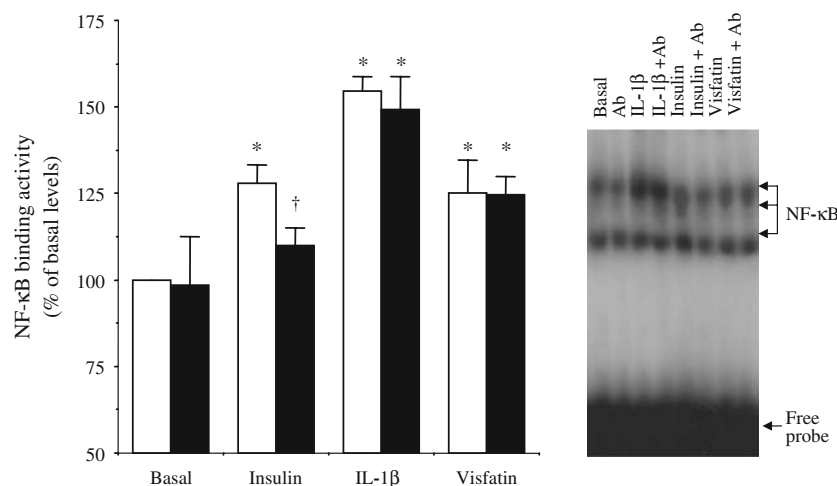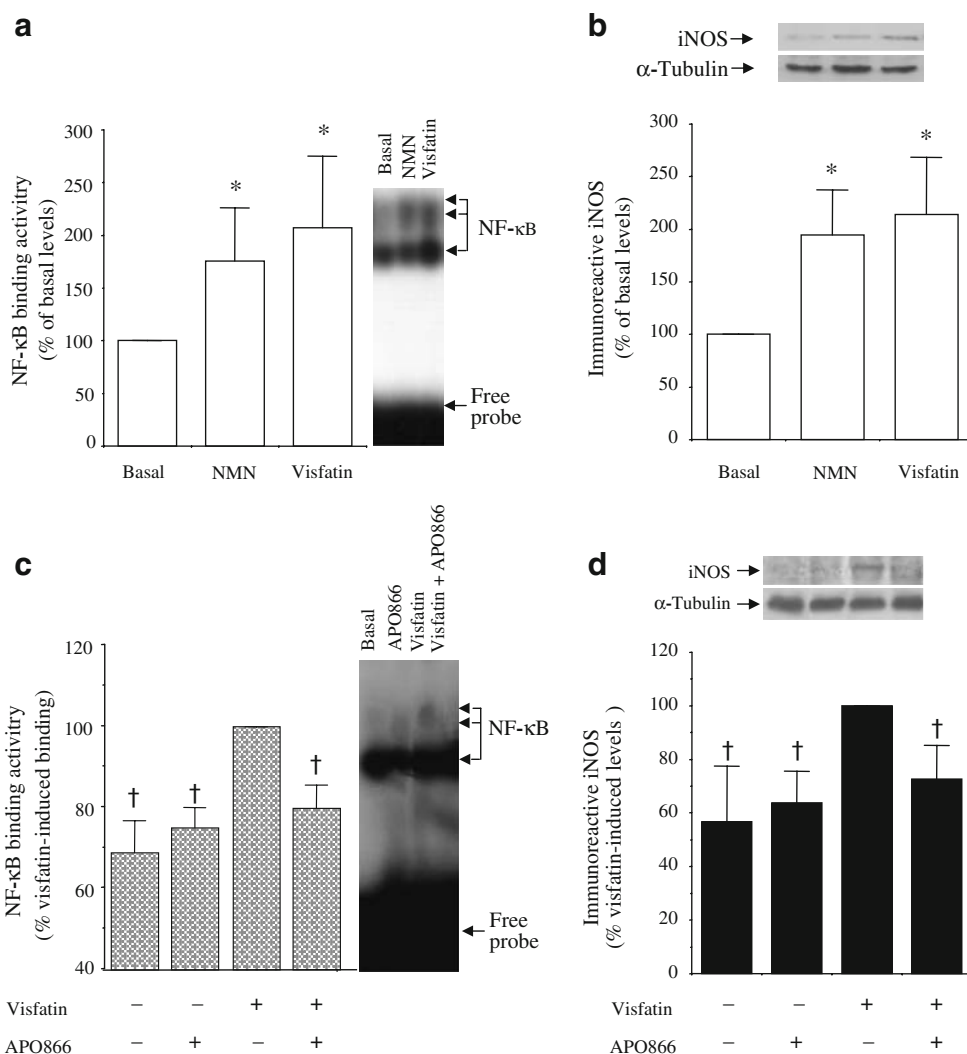
Fig. 3 NF-κB activation by ePBEF/NAMPT/visfatin in HASMC is not mediated by insulin receptor activation. HASMC were exposed for 18 h to insulin (10 nmol/l), IL-1β (10 ng/ml) or visfatin (100 ng/ml) and NF-κB was determined by electrophoretic mobility shift assay (white bars). In parallel experiments, HASMC were pre-incubated for 2 h with a commercial antibody (Ab) that blocks the insulin receptor (black bars). A representative gel is shown on the right. Means±SEM of at least three independent experiments. *$p<0.05$ vs basal; †$p<0.05$ vs matched treatment without antibody

Fig. 4 NAMPT activity mediates ePBEF/NAMPT/visfatin-induced NF-κB activation and iNOS induction. HASMC were exposed to either the NAMPT activity product NMN (100 μmol/l) or visfatin (100 ng/ml) and 18 h later NF-κB activation (**a**) and iNOS induction (**b**) were determined. In another set of experiments, HASMC were treated for 18 h with visfatin (100 ng/ml) in the presence or absence of the NAMPT activity inhibitor APO866 (100 nmol/l), after which NF-κB activation (**c**) and iNOS levels (**d**) were determined. Representative gels are shown. Means±SEM of at least three independent experiments. *$p<0.05$ vs basal; †$p<0.05$ vs visfatin alone

concentration of 50 ng/ml. Although the number of studies measuring plasma levels of ePBEF/NAMPT/visfatin is still limited, circulating levels of ePBEF/NAMPT/visfatin of 40–60 ng/ml have been reported in obese or type 2 diabetic patients, whereas their matched healthy controls showed lower levels [2, 3]. Such elevated levels of plasma ePBEF/NAMPT/visfatin have also been detected in patients with chronic kidney disease [5].

The current knowledge of the actions of PBEF/NAMPT/visfatin in vascular smooth muscle cells is still limited. On the one hand, intracellular PBEF/NAMPT/visfatin has been reported to induce maturation [16] and to extend the lifespan of human vascular smooth muscle cells by activating members of the sirtuin family and restraining the accumulation of p53 [17]. On the other hand, ePBEF/NAMPT/visfatin, released by different cells and tissues including the visceral and perivascular adipose tissue, can reach vascular smooth muscle and behave as a growth factor for this cell type [18]. In the present study, we have further identified ePBEF/NAMPT/visfatin as a pro-inflammatory factor for vascular smooth muscle cells.

A pro-inflammatory action of ePBEF/NAMPT/visfatin has been reported in non-vascular cell types and it has been related to different pathologies, such as arthritis, acute lung injury, inflammatory bowel disease or pre-term parturition [19, 20]. In the context of cardiovascular diseases, ePBEF/NAMPT/visfatin is seen as a new agent favouring vascular inflammation, a condition that plays a key role in the initiation and progression of atherosclerosis. Indeed, ePBEF/NAMPT/visfatin triggers the release of monocyte chemotactic protein-1, IL-6 and IL-8 and the activation of matrix metalloproteinases in human endothelial cells [21, 22]. Furthermore, an enhanced PBEF/NAMPT/visfatin content has been reported in human unstable carotid and coronary atherosclerotic plaques [23], as well as in the adipose tissue of patients with coronary artery disease, when compared with control individuals [24]. The ability of ePBEF/NAMPT/visfatin to activate and inflame human vascular smooth muscle demonstrated herein reinforces a potential pro-atherosclerotic action of the adipocytokine.

Although ePBEF/NAMPT/visfatin was initially claimed as an insulin mimetic acting through the insulin receptor and exhibiting glucose-lowering properties [1], such a statement was later on retracted and remains highly controversial [25]. On the one hand, Xie et al. [26] have reported that the regulation of glucose uptake, cell proliferation and type I collagen production in human osteoblasts by ePBEF/NAMPT/visfatin requires the activation of the insulin receptor. Furthermore, other cellular effects triggered by ePBEF/NAMPT/visfatin, including the secretion of IL-8 and TNF-α by human peripheral blood mononuclear cells [23] and the uptake of glucose in rat mesangial cells [27], also seem to involve the insulin

receptor. Other studies, however, have discarded a role for this receptor in mediating the actions of ePBEF/NAMPT/visfatin in a wide range of cell types, including vascular cells, macrophages and pancreatic beta cells [11, 14, 18, 28]. The results presented herein do not support a role for the insulin receptor in mediating the pro-inflammatory action of ePBEF/NAMPT/visfatin in human vascular smooth muscle cells. Indeed, blocking the activation of the insulin receptor did not interfere with NF-κB activation by ePBEF/NAMPT/visfatin or IL-1β, and only abrogated the effects of insulin. The activation of NF-κB by insulin at pathophysiological concentrations (10 nmol/l) [29] observed herein is in line with recent studies showing a vascular pro-inflammatory action of insulin through endothelial cell activation [30] and chemotaxis induction [31]. Nevertheless, our data indicate that insulin and ePBEF/NAMPT/visfatin exert their direct pro-inflammatory actions in vascular smooth muscle through distinct mechanisms.

In 2006, Revollo et al. [11] demonstrated that ePBEF/NAMPT/visfatin regulates insulin secretion in pancreatic beta cells through its intrinsic NAMPT enzymatic activity. NAMPT converts nicotinamide to NMN, which is then transformed into NAD by nicotinamide/nicotinic acid mononucleotide adenylyltransferase [12]. In vertebrates, intracellular NAMPT plays an essential role in the biosynthesis of NAD from nicotinamide and therefore in the regulation of many cellular processes [12].

Here, we show that exogenous NMN mimicked the effects of ePBEF/NAMPT/visfatin on iNOS induction and NF-κB activation in HASMC. In line with our observations, Wang et al. have recently reported that exogenous NMN, at the same concentration used in this study, mimics the proliferative action of ePBEF/NAMPT/visfatin in rat vascular smooth muscle [18]. Here, we demonstrate that extracellular NMN can also be considered as a pro-inflammatory agent for human vascular smooth muscle. Furthermore, the stimulatory action of ePBEF/NAMPT/visfatin on iNOS and NF-κB in HASMC was abolished upon co-incubation with the NAMPT activity inhibitor APO866. Taken together, these results strongly suggest that extracellular NMN generation through NAMPT activity is on the basis of the pro-inflammatory action of ePBEF/NAMPT/visfatin in human vascular smooth muscle. Such a mechanism of action also seems to account for the growth factor-like properties of ePBEF/NAMPT/visfatin in vascular smooth muscle cells [18]. NMN has been recently proposed to permeate mammalian cell membranes, possibly through NAD uptake mechanisms [11, 18, 32]. Therefore, the extracellular NMN generated trough NAMPT activity may enter the cell and then trigger the intracellular pro-inflammatory signals explored herein through mechanisms that remain to be elucidated.

In conclusion, our findings identify ePBEF/NAMPT/visfatin as an adipocytokine that can promote vascular smooth muscle cell inflammation. We propose that this effect is not mediated by the activation of the insulin receptor, but rather relies on the intrinsic NAMPT activity of the adipocytokine. We finally suggest that ePBEF/NAMPT/visfatin may have a direct role in vascular dysfunction and inflammation associated to metabolic disorders.

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