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The adipokine chemerin augments vascular reactivity to contractile stimuli via activation of the MEK-ERK1/2 pathway

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ABSTRACT

Aims: Cytokines interfere with signaling pathways and mediators of vascular contraction. Endothelin-1 (ET-1) plays a major role on vascular dysfunction in conditions characterized by increased circulating levels of adipokines. In the present study we tested the hypothesis that the adipokine chemerin increases vascular contractile responses via activation of ET-1/ET-1 receptors-mediated pathways.
Main methods: Male, 10–12 week-old Wistar rats were used. Endothelium-intact and endothelium-denuded aortic rings were incubated with chemerin (0.5 ng/mL or 5 ng/mL, for 1 or 24 h), and isometric contraction was recorded. Protein expression was determined by Western blotting.
Key findings: Constrictor responses to phenylephrine (PE) and ET-1 were increased in vessels treated for 1 h with chemerin. Chemerin incubation for 24 h decreased PE contractile response whereas it increased the sensitivity to ET-1. Endothelium removal significantly potentiated chemerin effects on vascular contractile responses to PE and ET-1. Incubation with either an ERK1/2 inhibitor (PD98059) or ETA antagonist (BQ123) abolished chemerin effects on PE- and ET-1-induced vasoconstriction. Phosphorylation of MEK1/2 and ERK1/2 was significantly increased in vessels treated with chemerin for 1 and 24 h. Phosphorylation of these proteins was further increased in vessels incubated with ET-1 plus chemerin. ET-1 increased MEK1/2, ERK1/2 and MKP1 protein expression to values observed in vessels treated with chemerin.
Significance: Chemerin increases contractile responses to PE and ET-1 via ERK1/2 activation. Our study contributes to a better understanding of the mechanisms by which the adipose tissue affects vascular function and, consequently, the vascular alterations present in obesity and related diseases.

Introduction

The white adipose tissue has been recognized as an endocrine organ that releases anti-inflammatory adipokines, like adiponectin, and pro-inflammatory adipokines, like tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and plasminogen activator inhibitor-1 (Ahima, 2006; Galic et al., 2010; Guerre-Millo, 2004; Trayhurn and Wood, 2005). The balance between the actions of these adipokines is affected in many cardiovascular diseases and seems to be implicated in the alterations of vascular function associated with these conditions (Weisberg et al., 2003; Xu et al., 2003; Matsuzawa, 2005).

Vascular reactivity to contractile/relaxant stimuli is one of the most important determinants of vascular tonus. Previous studies have shown that adipokines can potentially affect vascular reactivity (Klemm et al., 1995; Vicaut et al., 1996; Pickkers et al., 2002; Vila and Mercedes, 2005). Therefore, it is highly likely that adipokines might contribute to the genesis of vascular dysfunction by directly affecting vascular reactivity. In addition to the classical adipokines, a novel adipokine, termed chemerin, was recently discovered (Wittamer et al., 2003; Bozaoglu et al., 2007; Goralski et al., 2007; Roh et al., 2007). Chemerin was originally identified as a chemoattractant for immune cells such as macrophages and dendritic cells (Wittamer et al., 2004). However, chemerin receptor is also expressed in endothelial cells, and its expression is regulated by inflammatory cytokines, including TNF-α, IL-1β, and IL-6 (Kaur et al., 2010). Thus, it is likely that chemerin contributes to changes in vascular reactivity in inflammatory states. Accordingly, increased chemerin expression was demonstrated in adipocytes from mice fed a high fat diet (Roh et al., 2007). In addition, serum and adipose tissue chemerin levels are increased in women with metabolic syndrome (Bozaoglu et al., 2009). In humans, blood chemerin concentration seems to be associated with components of metabolic syndrome, such as body

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mass index, blood triglycerides, and blood pressure. Although chemerin is frequently present in conditions associated with altered vascular function, no studies have specifically assessed its vascular effects.

The endothelin-1 (ET-1) system contributes to vascular dysfunction in cardiovascular diseases, and, accordingly, circulating levels of ET-1 are increased in a variety of vascular diseases (Schiffrin, 2005; Carneiro et al., 2008; Weil et al., 2011). The effect of pro-inflammatory cytokines on ET-1 system is of great relevance, because in many situations where the levels of pro-inflammatory cytokines are elevated, the activity of ET-1 is also altered (Pasceri et al., 2000; Cooke and Oka, 2002; Verma et al., 2003; Molet et al., 2000). Increased levels of ET-1 are associated with augmented activation of MAPKs, especially ERK 1/2, a major signaling pathway activated by ET-1 in the vascular system (Kobayashi et al., 2008; Matsumoto et al., 2009).

Based on these observations, we hypothesized that chemerin augments vascular responses to contractile stimuli via activation of ET-1/ET-1 receptor-mediated pathways. To test our hypothesis, the effects of chemerin were determined in isolated aortic rings. The contribution of ET-1 to chemerin effects was assessed by pharmacological inhibition of the MEK-ERK1/2 pathway and ETA receptors as well as by molecular approaches.

Methods

Animals

All experimental protocols were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). Protocols were approved by the Committee for Animal Research of the University of Sao Paulo, Ribeirao Preto, Brazil (Protocol No. 10.1.1295.53.7/11) and by the Medical College of Georgia Committee on the Use of Animals in Research and Education. Ten to twelve week-old male Wistar rats (purchased from Harlan Laboratories, Indianapolis, IN) were used. The rats were maintained on a 12-hour light/dark cycle under controlled temperature (22 ± 1 °C) with access to food and water ad libitum.

Chemerin incubation procedures

After euthanasia, thoracic aortas were rapidly excised and cleaned from fat tissue in an ice-cold (4 °C) Krebs–Henseleit-modified solution [(in mM): 130 NaCl, 14.9 $NaHCO_3$, 4.7 KCl, 1.18 KH_2PO_4, 1.17 $MgSO_4 \cdot 7H_2O$, 5.5 glucose, 1.56 $CaCl_2 \cdot 2H_2O$, and 0.026 EDTA], gassed with 5% CO_2/95% O_2 to maintain a pH of 7.4. Aortic rings (2–3 mm in length) were mounted on 2 stainless-steel wires in standard organ chambers for isometric tension recording, as described previously (Giachini et al., 2008). Vessels were allowed to equilibrate for about 30 min in Krebs–Henseleit solution. After the stabilization period, arterial integrity was assessed first by stimulation of vessels with 120 mmol/L of KCl. After washing and a new stabilization period, endothelial function was assessed by testing the relaxant effect of acetylcholine (ACh, 1 μM) on vessels contracted with phenylephrine (PE, 1 μM). Aortic rings exhibiting a vasodilator response to ACh greater than 90% were considered endothelium-intact vessels. In experiments with endothelium-denuded vessels, aortic rings were subjected to rubbing of the intimal surface. Rings showing a maximum of 5% relaxation to ACh were considered without endothelium.

Arterial segments were incubated with either vehicle (phosphate buffer solution, PBS) or chemerin (0.5 ng/mL and 5 ng/mL) for 1 h to verify acute effects of the cytokine. In another set of experiments, aortas were functionally evaluated after incubations for 24 h with vehicle (PBS) or chemerin (0.5 ng/mL and 5 ng/mL) in Eagle minimum essential medium containing l-glutamine (1.0%), fetal bovine serum (FBS, 10.0%), penicillin (0.5%), and streptomycin (0.5%). Pilot studies were performed before these concentrations of chemerin were selected. Chemerin was tested at the concentrations: 0.05, 0.5, 5 and 50 ng/mL. Chemerin 0.05 ng/mL did not significantly affect contractile responses induced by PE and ET-1. Chemerin 50 ng/mL produced effects similar to those observed with 5 ng/mL. Previous studies have estimated that the plasma and serum concentrations of active chemerin are 3.0 and 4.4 nM, respectively, in humans and 0.6 and 0.5 nM, respectively, in mice (Zabel et al., 2006). Therefore, the concentration used in the present study corresponds to physiological levels of chemerin.

Vascular function studies

Cumulative concentration–response curves to PE (0.001–10 μM) and ET-1 (0.001–30 nM) were performed in aortic rings previously incubated with chemerin (0.5 ng/mL or 5 ng/mL, for 1 or 24 h) or vehicle. To determine whether chemerin effects were dependent on the endothelium, responses were also assessed in endothelium-denuded arteries.

To determine the involvement of MEK-ERK1/2 pathway on chemerin effects, concentration-effect curves to PE and ET-1 were performed either in the absence (control) or in the presence of the MEK1/2 inhibitor PD98059 (1 μM). The protocol was performed in both endothelium-intact and endothelium-denuded vessels incubated with chemerin for 1 or 24 h. Experiments were also performed with 10 μM PD98059 (data not shown), and similar results were observed when compared with those observed with 1 μM PD98059. The effects of another MEK/ERK inhibitor, the compound U0126, were also investigated and similar results were obtained when compared with PD98059 (data not shown).

In order to investigate if the increased contractile response to PE after incubation with chemerin was associated with an overall impairment of the ET-1 system, rather than a specific PE-mediated signaling pathway, concentration–response curves to PE were performed in arteries incubated with a selective ETA receptor antagonist (BQ-123, 0.1 μM). Tissues were incubated with the inhibitors for 30 min prior to the incubation with chemerin. Each preparation was tested with a single agent.

Western blotting

To test whether chemerin alters the activity of MEK1/2, ERK1/2, and MKP1, endothelium-intact vessels were incubated with 0.5 ng/mL chemerin or vehicle for 1 or 24 h, and the activation of these proteins was examined. After the incubation protocols were performed, vessels were frozen in liquid nitrogen and proteins were extracted (50 μg) and separated by electrophoresis on 8% polyacrylamide gels and transferred to nitrocellulose membranes. Nonspecific binding sites were blocked with 5% skim milk in Tris-buffered saline solution with Tween (0.1%) for 1 h at 24 °C. Membranes were incubated with antibodies (at the indicated dilutions) overnight at 4 °C. Antibodies were as follows: anti-MEK1/2 (1:250, Sigma), anti-phospho MEK1/2 (1:1000, Sigma), anti-ERK1/2 (1:1000, Cell Signaling), anti-phospho ERK1/2 (1:1000, Cell Signaling), anti MKP1 (1:1000, Sigma), and anti-β-actin (1:20000, Sigma). After incubation with secondary antibodies, signals were revealed by chemiluminescence, visualized by autoradiography and quantified densitometrically. Results were normalized to β-actin expression and expressed as units relative to the control.

Drugs

Phenylephrine, acetylcholine, PD98059, BQ-123, and endothelin-1 were purchased from Sigma Chemical Co (St. Louis, MO). Chemerin was purchased from R&D Systems, Inc (Minneapolis, MN).

Data analysis and statistical procedures

Contraction to PE and ET-1 is expressed as percentage of KCl-induced response. The individual concentration–response curves were fitted into a curve by non-linear regression analysis. pD_2 (defined as the negative logarithm of the EC_{50} values) and maximal response (E_{max}) were compared by *t*-tests or ANOVA, when appropriated. The Prism software, version 5.0 (GraphPad Software Inc., San. Diego, CA, USA) was used to analyze these parameters as well as to fit the sigmoidal curves. Data are presented as mean ± SEM. *N* represents the number of animals used. *p* Values less than 0.05 were considered significant.

Results

Chemerin potentiates PE-induced vasoconstriction

The sensitivity to PE was significantly increased in vessels treated with 0.5 ng/mL chemerin for 1 h ($pD_2 = 7.8 \pm 0.1$) when compared to vessels treated with either vehicle ($pD_2 = 6.8 \pm 0.05$), or 5 ng/mL chemerin ($pD_2 = 6.6 \pm 0.05$) ($n = 5$, Fig. 1A). However, incubation with chemerin for 24 h decreased the maximal vascular contractile response to PE (E_{max} in %, vehicle = 144.6 ± 2.6; chemerin 0.5 ng/mL = 100.4 ± 2.5; chemerin 5 ng/mL = 82.4 ± 3.6, $n = 5$, $p < 0.05$; Fig. 1B).

A negative modulatory role of the endothelium on chemerin effects was observed in vessels incubated with the adipokine for both 1 and 24 h. Incubation with chemerin for 1 h significantly potentiated the maximal vascular contractile responses to PE in endothelium-denuded vessels (E_{max} in %, vehicle = 105.3 ± 1.6; chemerin 0.5 ng/mL = 141.2 ± 1.9; chemerin 5 ng/mL = 141.0.4 ± 10.1, $n = 5$, $p < 0.05$; Fig. 1C). A similar effect was observed in vessels incubated with chemerin for 24 h (E_{max} in %, vehicle = 104.0 ± 3.1; chemerin 0.5 ng/mL = 133. ± 2.3; chemerin 5 ng/mL = 150.0.4 ± 7.5, $n = 5$, $p < 0.05$; Fig. 1D).

Chemerin potentiates ET-1-induced vasoconstriction

Chemerin incubation for 1 h augmented the vascular sensitivity to ET-1. Similar effects were observed in vessels incubated with both concentrations of chemerin (pD_2, vehicle = 9.2 ± 0.06; chemerin 0.5 ng/mL = 10.5 ± 0.1; chemerin 5 ng/mL = 10.2 ± 0.06, $n = 5$, $p < 0.05$; Fig. 2A).

Chemerin incubation for 24 h significantly increased the sensitivity to ET-1 in vessels treated with 0.5 ng/mL chemerin ($pD_2 = 7.8 \pm 0.1$), when compared to vessels treated with either vehicle ($pD_2 = 6.8 \pm 0.05$) or 5 ng/mL chemerin ($pD_2 = 6.6 \pm 0.05$) ($n = 5$, Fig. 2B).

In vessels without endothelium, both concentrations of chemerin (1 h-incubation) augmented the vasoconstrictor sensitivity to ET-1 (pD_2, vehicle = 9.6 ± 0.1; chemerin 0.5 ng/mL = 11.6 ± 0.03; chemerin 5 ng/mL = 11.3 ± 0.06, $n = 5$, $p < 0.05$; Fig. 2C). In vessels incubated with chemerin for 24 h, the potentiating effect on ET-1-induced contraction was observed only with the higher concentration (E_{max} in %, vehicle = 102.2 ± 6.6; chemerin 0.5 ng/mL = 119.3 ± 4.7; chemerin 5 ng/mL = 170.5 ± 9.2, $n = 5$, $p < 0.05$; Fig. 2D).

The vascular effects of chemerin are dependent on ERK1/2 activation

Incubation of vessels with the ERK1/2 inhibitor, the compound PD98059, 30 min before the incubation with chemerin (for 1 h), abolished the effects of this cytokine on PE-induced vasoconstriction (pD_2; vehicle = 6.7 ± 0.1; chemerin 0.5 ng/mL = 7.7 ± 0.1; chemerin 5 ng/mL = 6.6 ± 0.04; vehicle + PD98059 = 7.2 ± 0.05; chemerin 0.5 ng/

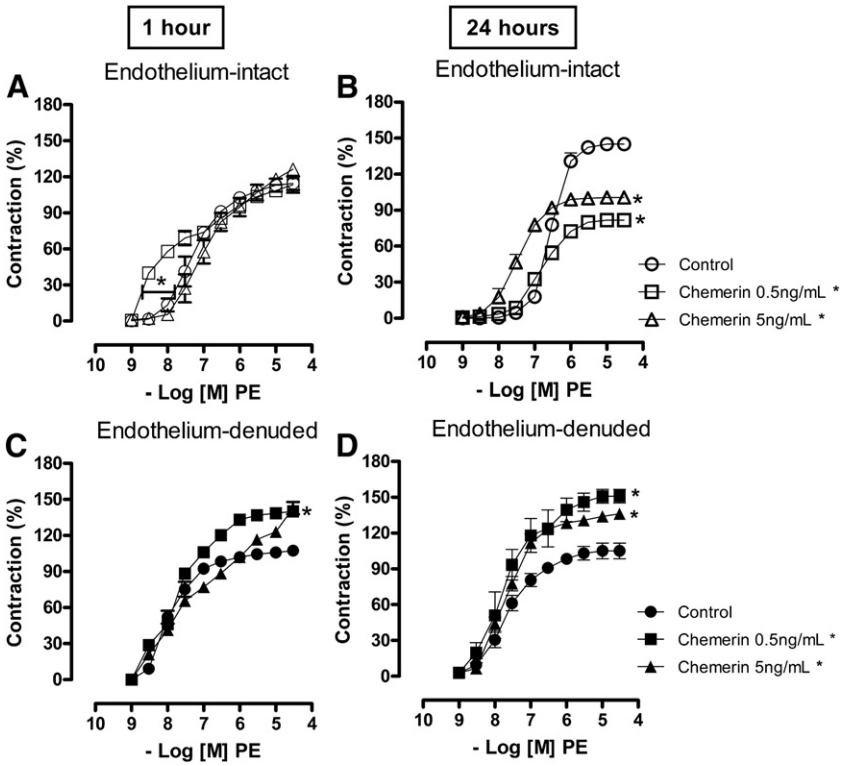

Fig. 1. Chemerin potentiates PE-induced vasoconstriction. The sensitivity to phenylephrine (PE) was increased in thoracic aortic rings treated for 1 h with 0.5 ng mL chemerin (A). Chemerin incubation for 24 h decreased the maximal vascular contractile response to PE (B). Removal of the endothelium significantly potentiated the maximal vascular contractile responses to PE after incubation with chemerin for 1 h (C). Similar effects were observed in vessels incubated with chemerin for 24 h (D). Experimental values of contraction were calculated relatively to the contractile response produced by 120 mmol/L KCl, which was taken as 100%. Results are presented as mean ± SEM for $n = 5$ in each experimental group. *, $p < 0.05$ vs. control (vehicle).

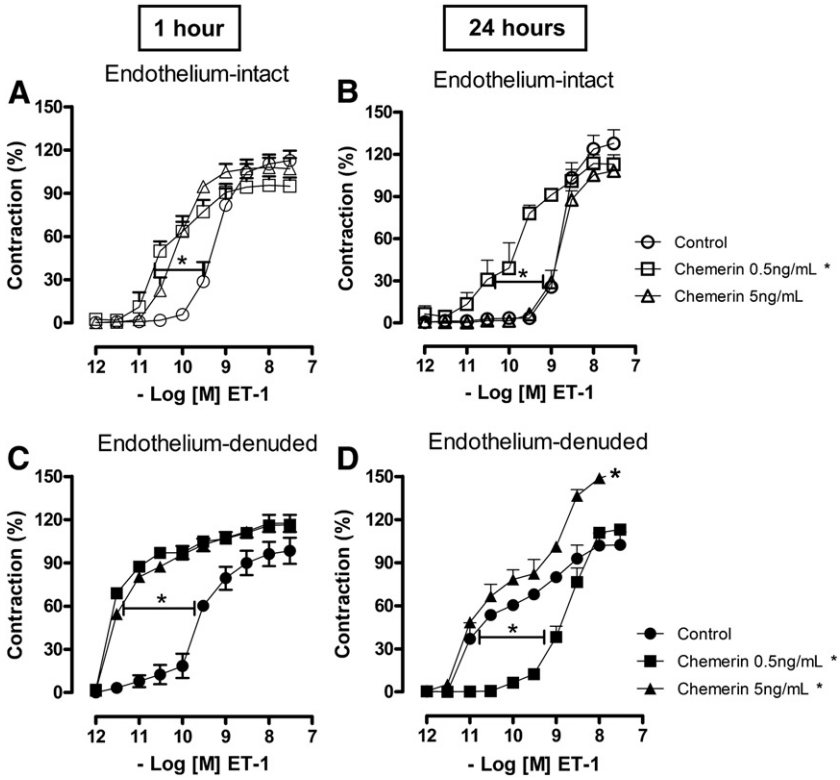

Fig. 2. Chemerin potentiates ET-1-induced vasoconstriction. Chemerin incubation for 1 h (A) and 24 h (B) augmented the sensitivity to endothelin-1 (ET-1). A negative modulatory effect of the endothelium was observed on the effects of chemerin on ET-1 responses. In vessels without endothelium, chemerin incubation for 1 h (C) and 24 h (D) further augmented the vasoconstrictor sensitivity to ET-1. Experimental values of contraction were calculated relatively to the contractile response produced by 120 mmol/L KCl, which was taken as 100%. Results are presented as mean ± SEM for $n = 5$ in each experimental group. *, $p < 0.05$ vs. control (vehicle).

mL + PD98059 = 5.8 ± 0.1; chemerin 5 ng/mL + PD98059 = 5.6 ± 0.1) ($n = 6$, Fig. 3A). The same effect was observed in endothelium-denuded vessels (E_{max} in %, vehicle = 105.2 ± 1.6; chemerin 0.5 ng/mL = 143.0 ± 2.1; chemerin 5 ng/mL = 140.0 ± 6.8; vehicle + PD98059 = 102.3 ± 1.4; chemerin 0.5 ng/mL + PD98059 = 119.3 ± 1.3; chemerin 5 ng/mL + PD98059 = 106.4 ± 4.2, $n = 6$, $p < 0.05$; Fig. 3B).

The ERK1/2 inhibitor also abolished the 24-hour effects of chemerin on ET-1-induced constriction in endothelium-intact arteries

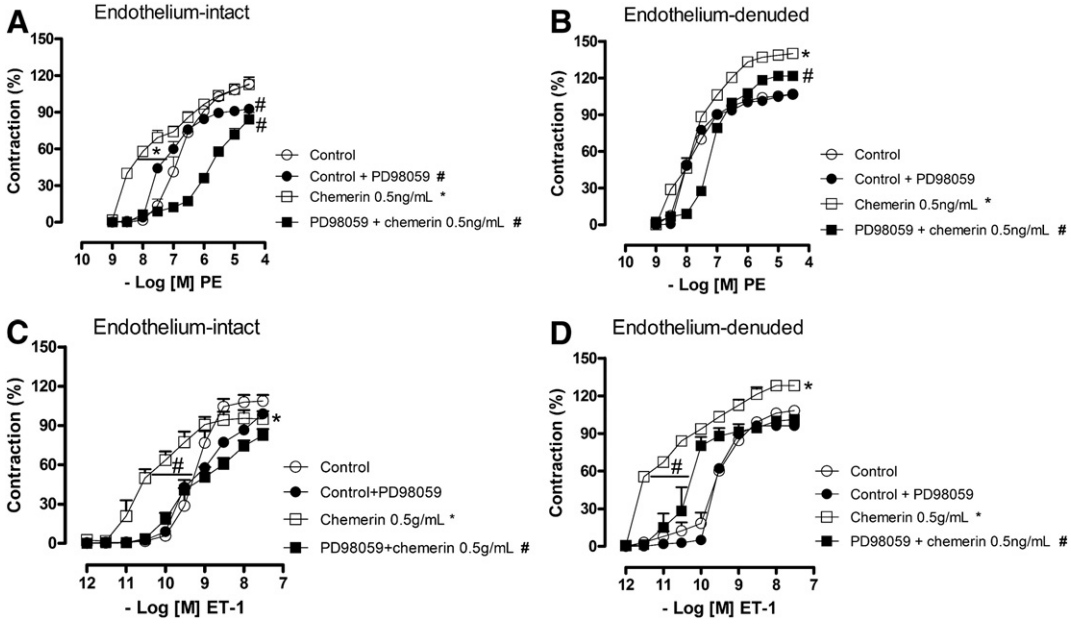

Fig. 3. The vascular effects of chemerin are dependent on ERK1/2 activation. The ERK1/2 inhibitor (PD98059), added 30 min before the incubation with chemerin (for 1 h), abolished chemerin effects on phenylephrine (PE)-induced contraction (A). The same effect was observed in endothelium-denuded vessels (B). ERK1/2 inhibition also abolished the 24 h-effects of chemerin on ET-1-induced vasoconstriction (C). PD98059 did not change the effects of chemerin in endothelium-denuded vessels (D). Experimental values of contraction were calculated relatively to the contractile response produced by 120 mmol/L KCl, which was taken as 100%. Results are presented as mean ± SEM for $n = 6$ in each experimental group. *, $p < 0.05$ vs. Control (vehicle); #, $p < 0.05$ vs. respective group in the absence of PD98059.

(pD_2, vehicle = 9.2 ± 0.1; chemerin 0.5 ng/mL = 10.2 ± 0.05; chemerin 5 ng/mL = 10.8 ± 0.1; vehicle + PD98059 = 9.2 ± 0.05; chemerin 0.5 ng/mL + PD98059 = 9.3 ± 0.1; chemerin 5 ng/mL + PD98059 = 9.4 ± 0.06, $p<0.05$; $n=6$, Fig. 3C). However, PD98059 attenuated but did not block chemerin effects in endothelium-denuded vessels (Fig. 3D).

ETA receptor activation mediates vascular effects of chemerin on PE responses

Chemerin effects on PE-induced vasoconstriction were abolished by the ETA antagonist BQ-123, (pD_2; vehicle = 6.7 ± 0.1; chemerin 0.5 ng/mL = 7.7 ± 0.1; chemerin 5 ng/mL = 6.6 ± 0.04; vehicle + BQ123 = 6.8 ± 0.05; chemerin 0.5 ng/mL + BQ123 = 7.0 ± 0.04; chemerin 5 ng/mL + BQ123 = 6.7 ± 0.05; $n=6$, Fig. 4A). Similar results were observed in endothelium-denuded vessels incubated with the ETA antagonist (E_{max} in %, vehicle = 103.0 ± 1.6; chemerin 0.5 ng/mL = 138.0 ± 1.9; chemerin 5 ng/mL = 140.2 ± 5.9; vehicle + BQ-123 = 105.6 ± 3.0; chemerin 0.5 ng/mL + BQ-123 = 90.07 ± 2.5; chemerin 5 ng/mL + BQ-123 = 94.7 ± 1.8, $n=6$, $p<0.05$; Fig. 4B).

Chemerin induces MEK-ERK1/2 pathway activation

Chemerin (0.5 ng/mL and 5 ng/mL for 1 h) increased vascular protein expression of phosphorylated MEK1/2 (Fig. 5A) and ERK1/2 (Fig. 5B), vs. vehicle (PBS)-treated aortic rings. ET-1 also increased vascular protein expression of phosphorylated MEK1/2 and ERK1/2 (values were similar to those observed in aortic rings treated with chemerin). MEK1/2 and ERK1/2 phosphorylation was further increased in vessels incubated with a combination of ET-1 and chemerin. Chemerin incubation for 1 h also increased vascular protein expression of the MAPK phosphatase 1 (MKP1), an enzyme that inactivates MAPK signaling ($n=4$, Fig. 5C).

Chemerin incubation for 24 h did not change the vascular protein expression of phosphorylated MEK1/2 (Fig. 5D) or ERK1/2 (Fig. 5E), vs. vehicle (PBS)-treated aortic rings. However, ET-1 increased vascular protein expression of phosphorylated MEK1/2 and ERK1/2. Similarly to the observed in vessels incubated for 1 h, MEK1/2 and ERK1/2 phosphorylation was further increased in vessels incubated with a combination of ET-1 and chemerin. The protein expression of the MKP1 was also increased after chemerin incubation for 24 h ($n=4$, Fig. 5F).

Discussion

The present study shows for the first time that the adipokine chemerin augments vascular responses to contractile stimuli and that ET-1-activated pathways contribute to the vascular changes produced by this cytokine. We also demonstrated that the vascular effects of chemerin occur through activation of MEK-ERK1/2 pathway.

Considering that cytokines activate several signaling pathways and mediators of vascular contraction (Klemm et al., 1995; Vicaut et al., 1996; Pickkers et al., 2002; Vila and Mercedes, 2005), and given that ET-1 plays a major role on vascular dysfunction in conditions characterized by increased circulating levels of adipokines (Schiffrin, 2005; Carneiro et al., 2008; Weil et al., 2011), we hypothesized that chemerin increases vascular responses to contractile stimuli via activation of ETA receptors and MEK-ERK1/2-mediated pathways. In fact, short term exposure (1 h) to chemerin augmented vasoconstriction induced by both the α1-adrenergic agonist PE and ET-1 in endothelium-intact vessels. The potentiating effect of chemerin on ET-1 responses was also observed after incubation for 24 h. Of relevance, chronic chemerin incubation did not produce consistent potentiation of vasoconstriction, which indicates that chemerin can activate additional pathways in a time-dependent manner. In addition, the endothelium seems to negatively modulate chemerin effects. This is supported by our findings that chemerin effects on PE and ET-1 contractions were greater in endothelium-denuded aortic rings.

Unlike the effects produced on ET-1 responses, chemerin incubation for 24 h (at the two concentrations) decreased the maximal vascular contractile response to PE. Considering that contractile responses are modulated by endothelium-derived factors and that the signaling pathways activated by PE differ from those activated by ET-1, it might be suggested that compensatory mechanisms are activated in these vessels to counteract chemerin effects on PE responses after prolonged periods of incubation. This hypothesis is further supported by the observation that in endothelium-denuded vessels, chemerin (at the two different concentrations, after 24 h of incubation) significantly potentiated the maximal vascular contractile response to PE. These data provide additional evidence that the endothelium negatively modulates the effects of the adipokine chemerin on PE effects in the vascular smooth muscle cells. In addition, our results also allow us to suggest that chemerin plays a major role influencing ET-1 responses, since its effects (even at low concentrations) in the contractile response induced by this agonist was not negatively modulated by the endothelium after 24 h incubation. To clarify whether chemerin effects on vascular contraction were dependent on the ET-1 pathway, concentration-effect curves to PE were performed in the presence of an ETA antagonist. Treatment with BQ-123 for 30 min previous to the incubation with chemerin abolished the enhanced vascular contractile response to PE. This result indicates that the ETA receptor subtype mediates the effects of chemerin and also adds an important insight into the role of ET-1 system on the effects of adipokines on vascular function.

Increased activation of signaling pathways involved in vasoconstriction may also contribute to the vascular effects of cytokines (Yamawaki, 2011). A key signaling pathway recruited by ET-1 receptor activation is the MAPK cascade (Bouallegue et al., 2007). Several reports have demonstrated that ET-1 activates MEK1/2, which in turn phosphorylates ERK1/2, promoting contraction of the vascular smooth muscle cells (Zubkov et al., 2000; Ishihata et al., 2002; Ansari et al., 2004). Although chemerin signaling pathways have not been well established, chemerin receptor (CMKLR1) activation is reported to increase the phosphorylation of

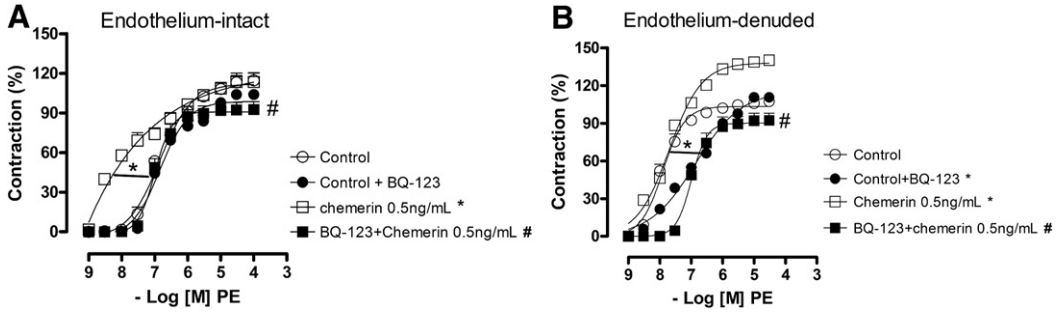

Fig. 4. ETA receptor activation mediates vascular effects of chemerin on PE responses. Chemerin effects on phenylephrine (PE)-induced vasoconstriction were abolished by the ETA antagonist, the compound BQ-123 both in endothelium-intact (A) and endothelium-denuded (B) vessels. Experimental values of contraction were calculated relatively to the contractile response produced by 120 mmol/L KCl, which was taken as 100%. Results are presented as mean ± SEM for $n=6$ in each experimental group. *, $p<0.05$ vs. Control (vehicle); #, $p<0.05$ vs. respective group in the absence of BQ123.

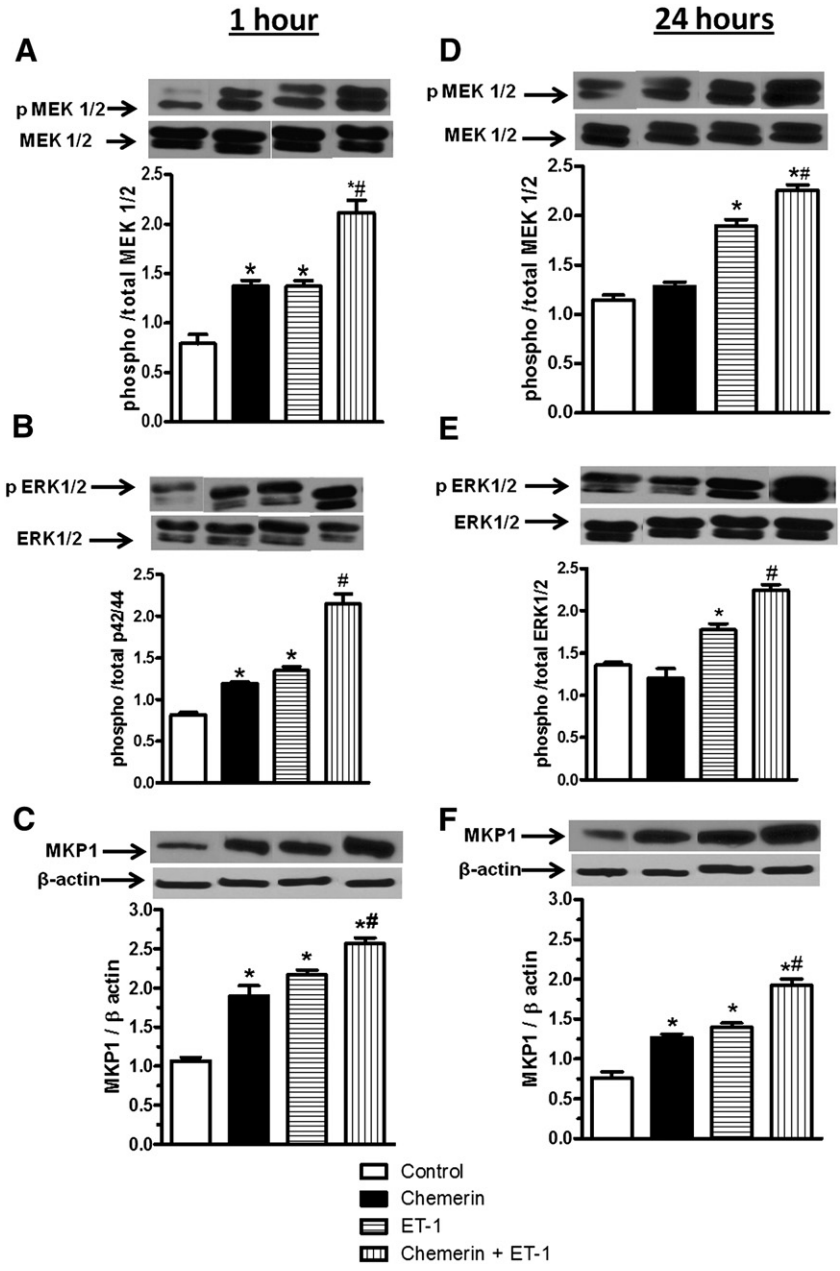

Fig. 5. Chemerin induces MEK-ERK1/2 pathway activation. Chemerin (0.5 ng/mL, for 1 h) and ET-1 (0.1 μM, for 10 min) increased vascular protein expression of phosphorylated MEK1/2 (A) and ERK1/2 (B). In addition, MEK1/2 and ERK1/2 phosphorylation was further increased in vessels incubated with a combination of ET-1 and chemerin. Chemerin incubation for 1 h also increased vascular protein expression of MKP1 (C). Chemerin incubation for 24 h did not change the vascular protein expression of phosphorylated MEK1/2 (D) or ERK1/2 (E). However, ET-1 increased the protein expression of phosphorylated MEK1/2 and ERK1/2. MEK1/2 and ERK1/2 phosphorylation was further increased in vessels incubated with a combination of ET-1 and chemerin. MKP1 protein expression was also increased after chemerin incubation for 24 h (F). Top, representative Western blotting images of (A) MEK1/2 proteins, (B) ERK1/2, and (C) MKP1. Bottom, corresponding bar graphs showing the relative expression of MEK1/2, ERK1/2, and MKP1 after normalization to β-actin expression. Results are presented as mean ± SEM for $n = 4$ in each experimental group. *, $p < 0.05$ vs. control (vehicle); #, $p < 0.05$ vs. Chemerin + ET-1. Endothelium-intact vessels were used in these experiments.

ERK1/2 (Wittamer et al., 2003). The relevance of this effect to the vascular actions of chemerin has not been described. In our study, inhibition of MEK1/2 with the compound PD98059 abolished the effects of chemerin on ET-1-induced vasoconstriction. Additionally, chemerin effects on PE-induced vascular responses also rely on this pathway. Similarly to those observed with ET-1, chemerin effects on PE-induced vasoconstriction were abolished by MEK1/2 inhibition, both in vessels with and without endothelium. This finding strongly suggests that activation of the MEK-ERK1/2 pathway contributes to chemerin effects on vascular reactivity.

To confirm that chemerin activates MEK1/2-mediated downstream responses, we determined the phosphorylation of these proteins. Western blotting analysis showed that chemerin (at 1 h) increased the phosphorylation of MEK1/2 and ERK1/2, a primary target of activated MEK1/2. In addition, chemerin potentiated ET-1-induced MEK1/2 and ERK1/2 phosphorylation. These data support our previous results showing that chemerin potentiates vasoconstriction to ET-1 by activating the MEK-ERK1/2 pathway. It is important to consider that although our results indicate that chemerin and ET-1 act through a common intracellular signaling transduction pathway, chemerin seems to activate additional mechanisms to potentiate vascular ET-1 responses. Increased activity of other kinases may be involved in these effects. Thus, potentiation of ET-1 signaling or even transactivation of ET-1-activated pathways by chemerin may provide an explanation for the potentiation of ET-1-induced MEK and ERK phosphorylation by chemerin.

We further probed the mechanisms leading to vascular activation of ERK1/2 by chemerin. MAPK pathway activation can be modulated at various steps, including receptor desensitization, dissociation of signaling complexes from receptor and deactivation of pathway mediators (Liu et al., 2007). Accordingly, phosphatases are important regulators of the MAPK pathway. MKPs are dual-specific phosphatases (DUSPs) that dephosphorylate tyrosine and threonine residues in MAPKs and thereby inactivate them (Boutros et al., 2008). MAPK phosphatase 1 (MKP1) is known to inactivate ERK1/2 (Boutros et al., 2008). Therefore, it is possible that chemerin increases ERK1/2 activation and the vasoconstrictor response to ET-1 by decreasing MKP1 activity. However, we observed that chemerin increased MKP1 levels, suggesting that chemerin regulates MKP1 expression as a negative feedback to modulate its effects on ERK1/2 activation.

Conclusions

Our study demonstrates that the adipokine chemerin potentiates the contractile response induced by ET-1 and PE, acting in both the endothelium and the vascular smooth muscle. A negative modulatory role of the endothelium on chemerin effects was also demonstrated. In addition, our data show that ETA receptor activation mediates chemerin effects on PE-induced response. Finally, we also showed that activation of the MEK-ERK1/2 pathway is involved on chemerin effects on ET-1 and PE-induced responses. These findings will contribute to a better understanding of how this cytokine can affect vascular reactivity in physiological and pathophysiological conditions.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.lfs.2012.04.013.

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