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Endothelin-1-induced arachidonic acid release by cytosolic phospholipase A_2 activation in rat vascular smooth muscle via extracellular signal-regulated kinases pathway

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Abstract

The present study investigates whether endothelin-1 (ET-1), like noradrenaline (NA), stimulates the release of arachidonic acid (AA) via cytosolic phospholipase A_2 ($cPLA_2$) in rat tail artery. In tail artery segments labelled with [^{3}H]AA, ET-1-induced AA release in a concentration-dependent manner with an EC_{50} of 1.3 nM. The effect of ET-1 was inhibited by bosentan and was insensitive to BQ788, suggesting the involvement of ET_A receptor. The stimulation of AA release induced by ET-1 was prevented by arachydonyl trifluoromethyl ketone ($AACOCF_3$), a selective inhibitor of $cPLA_2$ and not by RHC80267, a diacylglycerol lipase inhibitor. Furthermore, PD98059, inhibitor of mitogen-activated protein kinase kinase (MEK) cascade and calphostin C, a protein kinase C (PKC) inhibitor, prevented the stimulation of AA release induced by ET-1 and NA. Immunoblotting of the cytosolic fraction of rat tail arteries stimulated with ET-1 or NA showed an increase in extracellular signal-regulated kinases (ERKs) phosphorylation and this effect was abolished by calphostin C treatment. These findings show that in rat tail artery ET-1 and NA induce a sequential activation of protein kinase C and extracellular signal-regulated kinases that results in stimulation of AA release via $cPLA_2$ activation. This may represent a general pathway by which G-proteins coupled receptors stimulate AA release and its metabolites in vascular smooth muscle. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Endothelin-1; Noradrenaline; Cytosolic PLA_2; Arachidonic acid release; Extracellular signal-regulated kinases; Vascular smooth muscle

1. Introduction

Endothelins are a family of three peptides (endothelin-1, endothelin-2 and endothelin-3) that are implicated in the physiological control of vascular smooth muscle and myocardial contractility and growth [1–3]. ET-1 is involved in the development and maintenance of hypertension [4,5] in transgenic rats TGR (mREN2)27.

Endothelins act through specific G-protein-coupled receptors located in the cell surface. Two major endothelin receptors subtypes have been identified in mammalian tissues, namely ET_A and ET_B [1]. In the vascular bed, ET_A receptor is located on smooth muscle cells and mediates contraction, while ET_B receptor is present in endothelial cells and mediates the release of relaxing factors such as nitric oxide (NO) and prostacyclin. Binding of ET-1 to its receptors results in the increase of intracellular calcium and in the generation of a number of second messengers like inositol phosphates, DAG, AA and cyclic nucleotides (cAMP, cGMP). These second messengers activate enzymes, such as kinases and phosphatases, which in turn phosphorylate or dephosphorylate key cellular proteins, leading ultimately to the vascular responses of ET-1 [1–3,6]. In particular, AA and its metabolites (leukotrienes, prostaglandins, thromboxane, hydroxyeicosatetraenoic acids) appear to play a role in several important processes, including vascular smooth muscle contraction and growth [7–11].

It has been reported that ET-1 induces AA release through the activation of PLA_2 in vascular smooth muscle

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Abbreviations: AA, arachidonic acid; $AACOCF_3$, arachidonyl trifluoromethyl ketone; BSA, bovine serum albumin; $cPLA_2$, cytosolic phospholipase A_2; DAG, diacylglycerol; ERKs, extracellular signal-regulated kinases; ET-1, endothelin-1; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; NA, noradrenaline; PKC, protein kinase C; PLA_2, phospholipase A2; PLC, phospholipase C; PLD, phospholipase D; PSS, physiological salt solution.

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cells [12–14]. More recently it has been shown, in a wide variety of tissues and cells, that the agonist-induced AA release is mainly due to the activation of $cPLA_2$ [8,9,15–20]. PLA_2 represents a superfamily of esterases that hydrolyse the *sn*-2 ester bond releasing free fatty acids and lysophospholipids. A traditional classification is based on whether the PLA_2 is secreted from the cell ($sPLA_2$) or intracellular: Ca^{2+}-independent ($iPLA_2$) or Ca^{2+}-dependent cytosolic ($cPLA_2$) [15,21]. $sPLA_2$ isoforms require millimolar concentration of Ca^{2+} for activity, have low molecular masses (14–18 kDa), and demonstrate no selectivity for phospholipids containing AA. The $iPLA_2$s are located in both the cytosol and membrane fractions, do not require Ca^{2+} for activity and have molecular masses ranging from 29 to 85 kDa. $cPLA_2$ isoforms are found in the cytosol, have a higher molecular mass (85 kDa), require micromolar concentration of Ca^{2+} and are selective for AA containing phospholipids.

Activation of $cPLA_2$ by ET-1 has been demonstrated in non-vascular smooth muscle such as cat iris sphincter [22] and mesangial glomerular cells [23]. Although an ET-1 stimulated release of AA and its metabolites have been observed in vascular [12,13] and non-vascular [1,14] smooth muscle, direct evidence of $cPLA_2$ activation by ET-1 in this tissue has not been reported. $cPLA_2$ has been recently identified in rat caudal artery and it has been demonstrated that the stimulation of AA release induced by NA is due to an α_1-adrenergic receptor activation of $cPLA_2$ [24]. The regulation of $cPLA_2$ is not completely understood. Evidence has been accumulated that, besides micromolar Ca^{2+} concentration, phosphorylation of $cPLA_2$ is required for the stimulation of its enzymatic activity. In fact, several *in vitro* and *in vivo* studies have shown the involvement of ERKs in the receptor-mediated $cPLA_2$ activation [17,18,25,26]. ERKs are ubiquitous members of the MAPK, a family of serine/threonine kinases, which are activated in response to a variety of extracellular stimuli and are known to have a crucial role in cell proliferation [27,28]. Activation of MAPK cascade by ET-1 has been shown in rat mesangial cells [29] and in rat aortic smooth muscle cells [30,31]. The purpose of this study was to evaluate whether ET-1 stimulates AA release in smooth muscle of rat tail artery via $cPLA_2$. The involvement of protein kinases in the molecular mechanism of the receptor-promoted activation of $cPLA_2$ has also been investigated. The mechanism of ET-1-induced AA release in rat tail artery has been compared with that of NA. The results show that ET-1, as NA, activates $cPLA_2$ by signals transmitted through PKC and ERKs.

2. Materials and methods

2.1. Chemicals

[^{3}H]AA was purchased from NEN-DuPont. ET-1, noradrenaline and BQ788 were obtained from Sigma and were dissolved in twice distilled water. RHC80267 was obtained from Biomol and was dissolved in DMSO. Bosentan (Hoffmann-La Roche) was dissolved in twice distilled water. $AACOCF_3$, calphostin C, genistein, PD98059 and wortmannin were obtained from Calbiochem and were dissolved in DMSO. Antibodies anti-ERKs and p-ERKs were provided from Santa Cruz Biotechnology, Inc. and from New England Lab, respectively. All other chemicals were from Sigma. The final concentration of DMSO in the PSS was 0.1%.

2.2. Arachidonic acid release determination

AA release in rat tail arteries was determined as described by LaBelle and Polyak [24] with some modifications. Tail arteries from male Wistar rats were removed and rinsed with PSS, equilibrated with O_2, containing 140 mM NaCl, 5.9 mM KCl, 1.2 mM NaH_2PO_4, 1.4 mM $MgCl_2$, 2.5 mM $CaCl_2$, 5 mM HEPES, 11.5 mM glucose, pH 7.4. The arteries were dissected to remove fat and connective tissue and were cut into segments of 5 mm length. The endothelium was not removed except in some experiments performed with arteries in which endothelium was removed by perfusion with N_2 gas. After a preincubation (1 hr at 37°) in 3 mL PSS, the segments were incubated for 3 hr at 37° in 1 mL PSS containing 10 μg/mL BSA and 3 μCi [^{3}H]AA. The segments were then rinsed three times for 10 min in 4 mL of PSS, containing BSA (10 mg/mL) and twice with 4 mL of PSS, containing BSA (0.5 mg/mL). Finally, the segments (16 for one tail artery) were divided into groups of four segments each and transferred in 1 mL PSS containing BSA (0.5 mg/mL). Each group was considered as a sample and at fixed times was moved to fresh PSS using a customised container. In this way, it was possible to measure for each sample both basal and agonist-stimulated [^{3}H]AA release. In the experiments performed in the presence of bosentan, BQ788, $AACOCF_3$, RHC80267, PD98059 and wortmannin, 30 min incubation was carried out before the addition of the agonists (ET-1 or NA). In the experiments with genistein and calphostin C, 60-min incubation was performed before the addition of the agonists. Due to the photoactivable properties of calphostin C, the experiments with this substance were performed under fluorescence lighting. The radioactivity released by the artery segments in the PSS was measured by liquid scintillation counting. Total radioactivity incorporated was determined after dissolving the artery segments with Solvable (Packard). [^{3}H]AA release is expressed as % of released radioactivity related to the total radioactivity incorporated by the tissue.

2.3. Isolation of rat tail artery and cytosolic preparation

Tail arteries from male Wistar rats were dissected, cleaned of fat and connective tissue, opened longitudinally to remove endothelium and divided in two segments

(control and treated) of the same length. The segments were incubated for 60 min at 37° in PSS, equilibrated with O_2. Each segment was then transferred in 3 mL of PSS with or without the agonist (50 nM ET-1 or 50 μM NA) and incubated at 37° for 10 min. In the experiments performed in the presence of calphostin C (1 μM), a preincubation of 60 min with this inhibitor was carried out before the addition of the agonists. At the end of the incubation, the segments were removed from the medium, quickly frozen in liquid nitrogen, pulverised in a mortar and homogenised in 1 mL lysis-buffer (12.5 mM Tris, 2 mM EGTA, 25 mM β-glycerophosphate, 2 mM Na_3VO_4, 10 μM PMSF, 1 μM leupeptin, 5 μM aprotinin). The homogenate was then centrifuged for 10 min at 300 *g* and the supernatant (cytosolic fraction) was centrifuged at 24,000 *g* for 10 min. The cytosolic fraction obtained was frozen with liquid nitrogen and stored at −80° until use.

2.4. Protein assay

Protein concentration of the supernatant was determined with Bradford's method using BSA as standard.

2.5. Immunoblot analysis of ERKs expression and phosphorylation

Cytosolic fraction (40 μg) from rat tail artery was immunoblotted with rabbit polyclonal antibodies for ERKs and for p-ERKs detection, as previously described [32]. It was solubilised in Laemmli buffer and then separated by electrophoresis through a 10% polyacrylamide gel. Proteins separated on the gels were electroblotted onto nitrocellulose membrane (Hybond ECL, Amersham) in blotting buffer containing Tris 48 mmol/L, glycine 39 mmol/L, SDS 0.037%, methanol 20% (v/v) for 3 hr at 100 V in the cold, using a Transblot cell (Elettrofor). The membranes were blocked overnight at 4° in T-PBS containing PBS and 0.05% (v/v) Tween, and 5% BSA. Membranes were exposed to primary antibody (1:4000 dilution) for anti-ERKs, (1:1500 dilution) for anti-p-ERKs overnight at 4°. Membranes were washed (4× for 20 min) with the same buffer and then incubated with 1:4000 goat anti-rabbit antibody conjugate to horseradish peroxidase. Detection was made using the enhanced chemiluminescence system (ECL) from Amersham. Blots were scanned and quantified with a Bio-Rad Chemiluminescence Molecular Imaging Systems, and results were expressed relative to the control (s), on the same blot, set at 100%. To confirm the specificity of the signal detected with the phospho-antibody, the same blot was stripped using ReBlot Plus (Chemicon) and reprobed with control anti-ERK antibody.

2.6. Data analysis

Data of AA release shown in the figures are mean ± SEM of at least three independent experiments with triplicate or duplicate measurements. The statistical significance of the observed differences was analysed by paired *t* test, for the comparison between basal and agonist-stimulated AA release measured on the same artery segment; by unpaired *t* test, for the comparison of AA release between two different groups of arteries. $P < 0.05$ was considered to be statistically significant. The intersubject variability was analysed by one-way ANOVA comparing at least three different rats for each condition. No significant intersubject differences were found ($P > 0.05$). The concentration–response curve and the EC_{50} value were determined with GraphPad software. Data of ERKs phosphorylation are expressed as mean ± SEM of four independent experiments. The statistical significance of the observed differences was analysed by unpaired *t* test ($P < 0.05$ was considered to be statistically significant).

3. Results

3.1. Effect of ET-1 on [^{3}H]AA release

ET-1 significantly stimulates AA release from rat tail artery. The stimulation was observed after 1 min of treatment and the maximal increase was reached at 10 min. This time was chosen in all the experiments reported. Preliminary experiments showed that ET-1-induced AA release was not affected by the presence of endothelium, as observed by LaBelle and Polyak for NA [24]. Therefore, all the experiments of AA release determination have been performed on rat tail arteries with endothelium.

ET-1 increased AA release in a concentration-dependent manner with an EC_{50} (concentration producing half-maximal response) value of 1.3 nM. A significant increase ($P < 0.05$) was observed at 0.5 nM, and a maximal stimulation was observed at 10 nM (Fig. 1). The concentrations of ET-1 used in all the experiments were above the EC_{50} value.

3.2. Effect of ET-1 receptor antagonists on ET-1-induced AA release

To investigate the receptor involved in the stimulation of AA release induced by ET-1, rat tail artery segments were preincubated with BQ788 (a selective antagonist of ET_B receptor) or with bosentan (an ET_A-ET_B antagonist). As shown in Fig. 2, 1 μM BQ788 was devoid of any effect on AA release induced by 20 nM ET-1 while 1 μM bosentan completely prevented it. This result strongly indicates that ET_A receptors are involved in ET-1 stimulated AA release in rat tail artery.

3.3. Effect of RHC8026 and AACOCF$_3$ on ET-1- and NA-induced AA release

AA can be released from membrane phospholipids either through a direct action of $cPLA_2$ or via the stimulation of

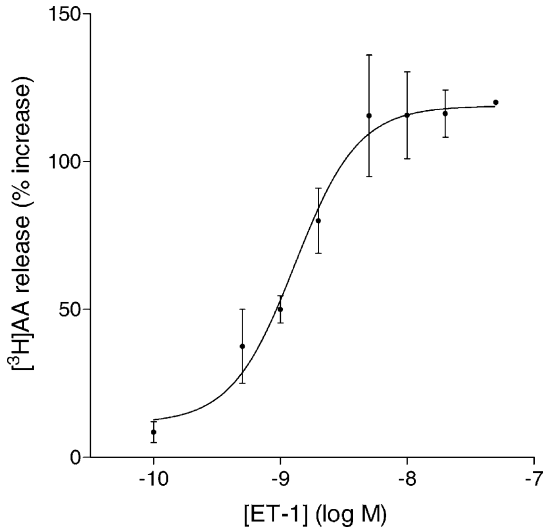

Fig. 1. Concentration–response curve for [^{3}H]AA release induced by ET-1 in rat tail artery. Rat tail artery segments were labelled and incubated in PSS with the indicated concentrations of ET-1 as described in Section 2. Data are expressed as % increase of [^{3}H]AA release after 10 min incubation with ET-1, related to basal release. Basal [^{3}H]AA release, expressed as % radioactivity released in the medium related to total radioactivity (71.000 ± 12.000 cpm) incorporated by the tissue, was 1.5 ± 0.06% ($n = 20$) after 10 min incubation. Data are mean ± SEM of at least three independent experiments.

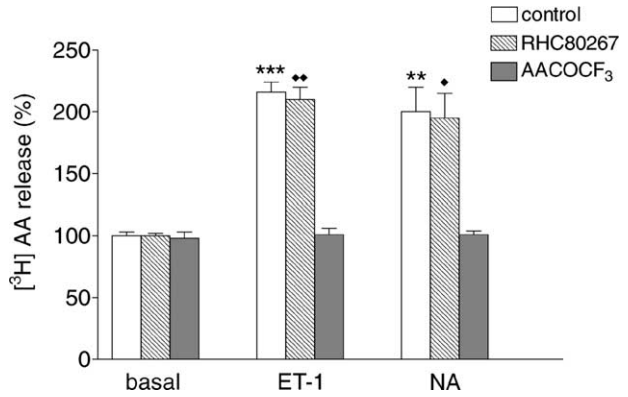

Fig. 3. Effect of RHC80267 and AACOCF$_3$ on [^{3}H]AA release induced by ET-1 and NA in rat tail artery. Rat tail artery segments were labelled and incubated in PSS as described in Section 2. Segments were incubated with or without 20 μM RHC80267 or 50 μM AACOCF$_3$ for 30 min and then stimulated with 20 nM ET-1 or 20 μM NA for 10 min. The inhibitors were also included when the arteries were stimulated with the agonists. Basal [^{3}H]AA release (without any inhibitor) was 1.8 ± 0.05% of total radioactivity incorporated. Data are mean ± SEM of four independent experiments. In control arteries, $^{***}P < 0.001$ (ET-1 vs. basal) and $^{**}P < 0.01$ (NA vs. basal); in RHC80267-treated arteries, $^{◆◆}P < 0.01$ (ET-1 vs. basal) and $^{◆}P < 0.05$ (NA vs. basal).

PLC or PLD followed by DAG lipase [33]. It has been observed that the treatment of rat tail artery segments with the DAG lipase inhibitor RHC80267 had no effect on AA release stimulated by NA while AACOCF$_3$, a selective inhibitor of cPLA$_2$, prevented it [24]. The effect of 20 μM RHC80267 and 50 μM AACOCF$_3$ on AA release stimulated by 20 nM ET-1 or 20 μM NA is shown in Fig. 3. ET-1-induced AA release, is unaffected by RHC80267 and completely prevented by AACOCF$_3$. The same result is obtained in artery segments stimulated with NA, confirming the previous observation of LaBelle and Polyak [24]. This result indicates that, like NA, the ET-1-induced AA release is mediated by cPLA$_2$ activation.

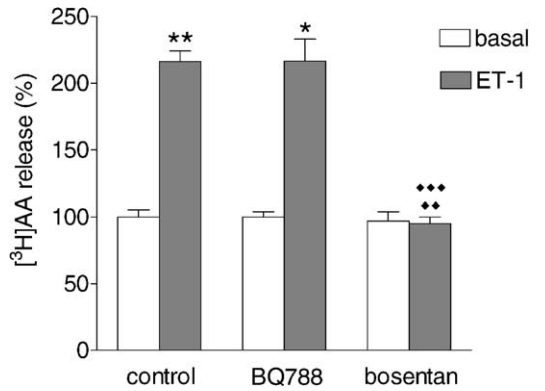

Fig. 2. Effect of BQ788 and bosentan on [^{3}H]AA release induced by ET-1 in rat tail artery. Rat tail artery segments were labelled and incubated in PSS as described in Section 2. Segments were incubated with or without 1 μM BQ788 or 1 μM bosentan for 30 min and then stimulated with 20 nM ET-1 for 10 min. Basal [^{3}H]AA release (without any antagonist) was 1.6 ± 0.08% of total radioactivity incorporated. Data are mean ± SEM of three independent experiments. $^{**}P < 0.01$ and $^{*}P < 0.05$ (basal vs. stimulated), $^{◆◆◆}P < 0.001$ (ET + bosentan vs. ET control) and $^{◆◆}P < 0.01$ (ET + bosentan vs. ET + BQ788).

3.4. Effect of protein kinases inhibitors on ET-1- and NA-induced AA release

The effect of some protein kinases inhibitors on ET-1- and NA-induced AA release in rat tail artery are shown in Fig. 4. Genistein (5 μM), an inhibitor of protein tyrosin kinases, and wortmannin (0.1 μM), an inhibitor of phosphatidylinositol-3-kinase, had no effect on the stimulation of AA release induced either by 20 nM ET-1 or 20 μM NA. On the other hand, calphostin C (1 μM), an inhibitor of PKC, and PD98059 (100 μM), an inhibitor of MEK cascade, completely prevented AA release.

3.5. Effect of ET-1 and NA on ERKs phosphorylation in rat tail artery

We studied the effect of ET-1 (50 nM for 10 min) and NA (50 μM for 10 min) on ERKs phosphorylation in rat tail artery segments treated or not with 1 μM of calphostin C. Fig. 5A and B represents a typical blot for ERK1 and ERK2 phosphorylation and expression in cytosolic fraction from rat tail artery, respectively. Densitometric analysis shows that ET-1 and NA significantly enhanced ERK1 and ERK2 phosphorylation ($P < 0.01$) and these effects were completely abolished by calphostin C treatment (Fig. 5C).

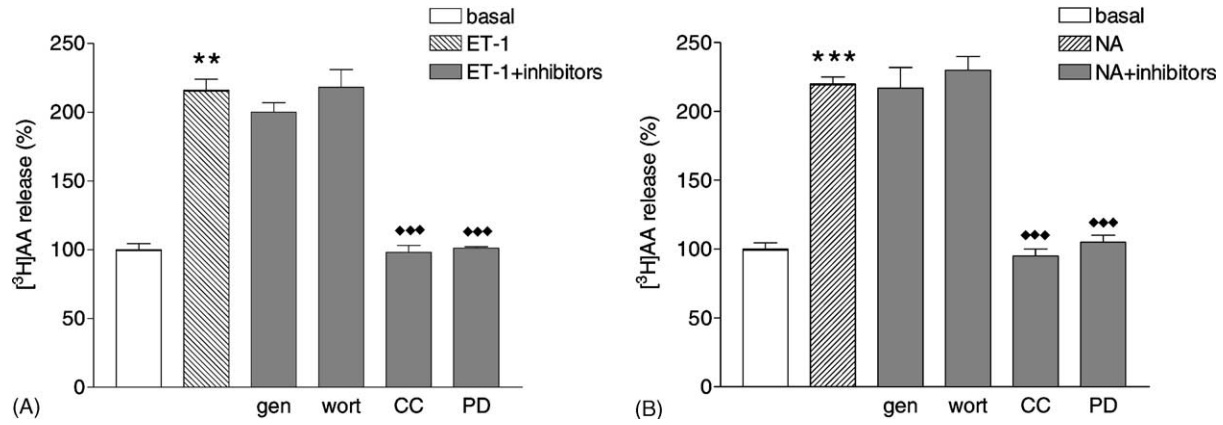

Fig. 4. Effect of genistein (gen), wortmannin (wort), calphostin C (CC) and PD98059 (PD) on [^{3}H]AA release induced by ET-1 (A) and NA (B). Rat tail artery segments were labelled as described in Section 2 and were incubated with the inhibitors: 5 μM genistein for 60 min, 100 nM wortmannin for 30 min, 1 μM calphostin C for 60 min or 100 μM PD98059 for 30 min. Afterwards the segments were stimulated with 20 nM ET-1 or 20 μM NA for 10 min. The inhibitors did not affect basal [^{3}H]AA release (not shown) and were also included, when the arteries were stimulated with the agonists. Basal [^{3}H]AA release (without any inhibitor) was 1.6 ± 0.07% of total radioactivity incorporated. Data are mean ± SEM of three independent experiments. (A) $^{**}P < 0.01$ (ET-1 vs. basal), $^{◆◆◆}P < 0.001$ (CC, PD vs. ET-1); (B) $^{***}P < 0.001$ (NA vs. basal), $^{◆◆◆}P < 0.001$ (CC, PD vs. NA).

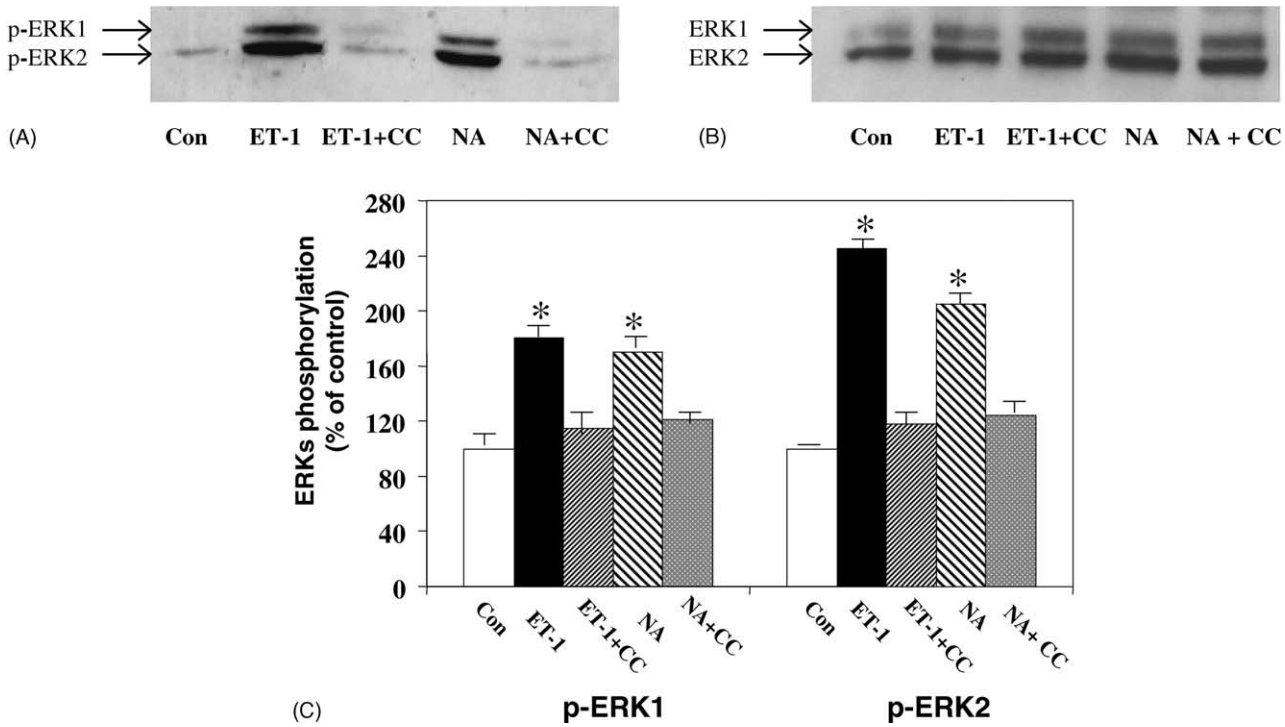

Fig. 5. Phosphorylation of ERKs in rat tail artery. Rat tail artery was treated with ET-1 (50 nM for 10 min) and NA (50 μM for 10 min) with or without preincubation for 60 min with 1 μM calphostin C (CC). Tissue homogenates were processed for ERK1 and ERK2 by immunoblotting with a polyclonal antibody that specifically recognises the phosphorylated forms of ERK1 and ERK2 (A); control arteries (Con). The same blot was stripped and reprobed with control anti-ERK antibody showing equal loading and confirming the specificity of the signal detected with the phospho-antibody (B). The ERK1 and ERK2 phosphorylation was quantified by densitometry and presented as mean ± SD from four independent experiments (C). ERK1 and ERK2 phosphorylation are expressed as a percentage relative to the control (means ± SD). $^{*}P < 0.01$ compared to the control (Con) and to the calphostin C effects (ET + CC and NA + CC, respectively).

4. Discussion

The present results show that in rat tail artery smooth muscle, ET-1 induces AA release through cPLA$_2$ activation. This effect occurs in the range of concentrations that induce the activation of PLC and the contraction of vascular smooth muscle [1,3,34]. The activation of cPLA$_2$ by ET-1 is indicated by the observation that AACOCF$_3$, a selective inhibitor of cPLA$_2$ [35], completely prevents the stimulation of AA release. The action of ET-1 is similar to that of NA, in agreement with the observation of LaBelle and Polyak [24] that in rat tail

artery NA-induced AA release is mediated by $cPLA_2$. AA can also be released from membrane phospholipids via the stimulation of PLC or PLD followed by DAG lipase activation [33]. Since the DAG lipase inhibitor RHC80267 does not affect the stimulation of AA release induced by ET-1 and NA [24], a pathway involving DAG lipase is likely excluded in rat tail artery. The lack of involvement of DAG lipase on ET-1-induced AA release has also been shown in human pericardial smooth muscle cells [14]. Our findings are in agreement with the observation that in many tissues [16–20], including vascular smooth muscle [8,9,24,36], agonist-induced AA release is mainly mediated by $cPLA_2$. Previous observations have shown that ET-1 stimulates $cPLA_2$ activity in non-vascular smooth muscle [22,23]. In contrast, the ET-1-induced PGI_2 formation in rat aorta is mediated through the activation of PLD and not $cPLA_2$ [37].

Concerning the receptor involved in $cPLA_2$ activation, our study suggests that ET_A receptor mediates the release of AA since BQ788, an ET_B selective antagonist, is ineffective on ET-1-induced AA release. This observation is in agreement with the results obtained by others in non-vascular smooth muscle cells: Wu-Wong *et al*. [14] showed that the ET_A receptor stimulates AA release through activation of PLA_2 in human pericardial smooth muscle cells while Schramek *et al*. [23] and Husain and Abdel-Latif [38] showed an ET_A-mediated stimulation of $cPLA_2$ on rat glomerular mesangial cells and cat iris sphincter smooth muscle, respectively. Although ET_B receptors are present in rat tail artery endothelium, the above reported lack of effect of BQ788 on ET-1-induced AA release indicates that only the ET_A receptors of vascular smooth muscle are involved in the stimulation of AA release.

The agonist-induced activation of $cPLA_2$ is regulated by two cellular events: the increase of intracellular Ca^{2+} concentration, which allows the enzyme translocation from cytosol to the membrane [18,39], and $cPLA_2$ phosphorylation [18–20,25]. Several studies have demonstrated the involvement of MAPK cascade in enhancing $cPLA_2$ activity [17–20,25,26,40]. In particular, two members of the MAPK family, ERK1 (p44MAPK) and ERK2 (p42MAPK) seem to be involved in agonist-induced $cPLA_2$ activation [17,18,25,26]. Furthermore it has been demonstrated, in non-vascular smooth muscle cells, that $cPLA_2$ phosphorylation may be also due to the activation of p38MAPK [22,41]. Several hypotheses have been put forward, in addition to ERK-mediated activation, to explain the molecular mechanism of $cPLA_2$ phosphorylation. Other kinases, like phosphatidylinositol-3-kinase, PKC and calcium/calmodulin-dependent kinase II (Ca/CaMK II), are reported to be responsible of $cPLA_2$ activation either directly or indirectly, promoting the ERK cascade [8,17,18,25,39]. Some studies propose that a protein tyrosin kinase [42,43] could be involved in the receptor-promoted phosphorylation of $cPLA_2$. This indicates that the pathways leading to $cPLA_2$ phosphorylation are different in different tissues and depend on the type of agonist studied.

Our data suggest that the sequential activation of PKC and ERKs, is a relevant pathway of ET-1 and NA mediated $cPLA_2$ stimulation in rat tail artery. In fact, both agonists promoted an increase of ERK1 and ERK2 phosphorylation and the treatment of rat tail arteries with PD98059, a selective inhibitor of MEK (ERK kinase), completely prevented the stimulation of AA release. Furthermore, the PKC inhibitor calphostin C prevented the ERK1/ERK2 phosphorylation and AA release induced by both ET-1 and NA. The involvement of PKC in ET-1-induced AA release has been shown in human pericardial smooth muscle cells [14].

In conclusion, the present data indicate that in rat tail artery smooth muscle ET-1, by means of an ET_A-receptor activation, induces AA release through the stimulation of $cPLA_2$, as previously observed for NA. Concerning the molecular mechanism of $cPLA_2$ activation, a common signal transduction pathway, that involves a sequential activation of PKC and ERKs, seems to be triggered by ET-1 and NA in this tissue. Studies on the regulation of ET-1-induced AA release may contribute to a better understanding of the effects of ET-1 on vascular smooth muscle contractility and growth.

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