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Role of mitogen-activated protein kinases in endothelin ET_B receptor up-regulation after organ culture of rat mesenteric artery

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Abstract

Organ culture of isolated arteries results in increased levels of endothelin ET_B (ET_B) receptor mRNA and in enhanced ET_B receptor mediated contraction. The present study was designed to pinpoint the mitogen-activated protein kinase (MAPK) subtype involved in up-regulation of ET_B receptors after organ culture of rat mesenteric arteries. Western blot and selective antibodies towards constitutional and phosphorylated MAPKs revealed the appearance of phosphorylated MAPK of the extracellular signal-regulated kinases (ERK) 1/2 type at 3 h of organ culture. The functional ET_B receptor and its mRNA expression were up-regulated after 24 h of organ culture. Following incubation with the MEK 1/2 specific inhibitor SB408039 or the raf inhibitor SB386023b the up-regulation was attenuated both for ET_B receptor responses and in ET_B receptor mRNA expression in the vessel segments. Neither Western blot nor myograph or mRNA analysis showed involvement of the other MAPKs studied. Our results suggest that the ERK1/2 MAPKs are involved in the endothelin ET_B receptor up-regulation following organ culture.

Keywords: MAP Mitogen-activated protein (kinase); ERK (Extracellular signal-regulated kinase)1/2; Sarafotoxin 6c; Endothelin; ET_B receptor

1. Introduction

Endothelin-1 (ET-1) is a vasoactive peptide produced in the endothelium of blood vessels and is one of the most potent contractile agonists known. Its actions are mediated by two G-protein coupled receptors, the endothelin ET_A and the ET_B receptors (Masaki, 1994). The endothelin ET_A receptor is a contractile receptor situated on the smooth muscle cells of the vessels whereas the endothelin ET_B receptor is mainly found on the endothelium, mediating dilatation (Szok et al., 2001). In addition, there is a small population of contractile endothelin ET_B receptors expressed on the smooth muscle cells in some arteries and veins (Uddman et al., 1999).

It has previously been revealed that contractile endothelin ET_B receptors on vascular smooth muscle cells are up-regulated via de novo transcription following organ culture (Adner et al., 1998a; Möller et al., 1997) and experimental focal cerebral ischemia (Stenman et al., 2002). However, the intracellular signalling pathways responsible for this up-regulation remain elusive. We have evidence for the involvement of protein kinase C (PKC) in the up-regulation both in cerebral and mesenteric arteries following organ culture (Hansen-Schwartz et al., 2002a; Uddman et al., 2002). Furthermore, cytokines may increase the efficacy of the endothelin ET_B receptor induced contraction (Uddman et al., 1999) via enhanced translation (Leseth et al., 1999; White et al., 1999).

The mitogen-activated protein kinases (MAPK) are a group of serine/threonine kinases that play an important role in the intracellular signalling in response to extracellular stimuli (Hazzalin and Mahadevan, 2002). The different MAPKs are involved in such important cellular functions as proliferation, differentiation and survival. There are three major MAPK pathways in mammals: the extracellular signal-regulated kinases (ERK1/2), the p38 MAPK and the c-*Jun*-N-terminal kinase (JNK) (Lewis et al., 1998). Of these, the ERK1/2 pathway is activated by mitogenic stimulation whereas the JNK and the p38 are so-called stress-activated

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protein kinases (SAPK), activated by various stress stimuli, for example cytokines (Irving and Bamford, 2002). Another kinase involved in proliferation is the serine/threonine kinase Akt/protein kinase B. The Akt cascade is activated by receptor tyrosine kinases, cytokine receptors and some G-protein coupled receptors, and has a role in vascular homeostasis and angiogenesis (Shiojima and Walsh, 2002).

The aim of the present study was to elucidate the involvement of MAPKs in organ culture induced ET receptor alterations. This was examined by incubating mesenteric arteries from rats for 24 h with different MAPK inhibitors. Functional studies were performed using a sensitive in vitro system where ET-1 (endothelin ET_A and ET_B receptor agonist) and sarafotoxin 6c (S6c; selective endothelin ET_B receptor agonist) were used to induce endothelin receptor mediated contractile responses. To measure if MAPK inhibitors affected the mRNA levels of the ET receptors, we used real-time PCR. Western blot was used to confirm the presence of activated (phosphorylated) MAPKs in the vessels.

2. Materials and methods

2.1. Tissue preparation and organ culture procedure

A distal branch of the rat superior mesenteric artery (outer diameter < 1 mm) was chosen as experimental target since previous studies have shown that endothelin ET_B receptors are up-regulated in this vessel following 24 h of incubation with Dulbecco's modified Eagle's medium (DMEM) (Adner et al., 1998a; Möller et al., 2002). Male Wistar-Kyoto rats (200–350 g, M&B, Denmark) were anaesthetized with CO_2 and killed by decapitation. The artery was removed, dissected free from adherent tissue and cut into 1-mm-long circular segments. Segments were placed in wells containing 1 ml of DMEM, supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml), and incubated at 37 °C in humidified 5% CO_2 in air (pH 7.4). Paired segments were incubated in 10^{-5} M of the MAPK inhibitors PD98059, SB239063, SB203580, SB408039 or SB386023b.

Segments to be used for Western blots and real-time PCR were cultured for 0.5, 1, 2, 3 or 24 h, snap frozen in liquid nitrogen and subsequently stored at −80 °C until use. Segments to be used for in vitro pharmacology were incubated for 24 h before they were studied pharmacologically. To study the effect of MAPK inhibitors on the ET receptor up-regulation, the respective MAPK inhibitor was present in the medium during the entire incubation period, but not during the myograph tests.

2.2. Western blot

The tissue used for Western blots was incubated initially in 500 μl Laemmli solution and homogenized with a polytron (IKA Labortechnik, Germany). The lysates were centrifuged at 5000 × *g* for 5 min at 4 °C and the supernatant transferred to new tubes. Further Laemmli solution was added to obtain a concentration of approximately 75 mg tissue/ml. The lysates were stored at −20 °C until use.

The proteins were heated to 97–100 °C for 3–5 min to denature most proteins. Equal amounts of the boiled supernatant fraction were loaded onto a 10% polyacrylamide gel, separated by electrophoresis (Mini-protean 3, Bio-Rad, CA) and then transferred to semi-blot PVDF membranes (Bio-Rad) by semidry blotting (Trans-blot SD, Bio-Rad). The Western blots were blocked with a solution of 5% skim milk in TBS-T (0.1% Tween-20 in 1 l H_20 with 24.2 g TRIsma base and 80 g NaCl) and incubated overnight at 4 °C in TBS-T containing skim milk and an appropriate dilution of polyclonal antibodies. The membranes were washed in TBS-T, incubated for 90 min with anti-rabbit IgG horseradish peroxidase-labelled secondary antibody (1:50,000). After further washing, the PVDF membranes were subjected to SuperSignal West and the chemiluminescence was detected by a Luminiscent Image Analyzer (Fujifilm Science Imaging systems, Japan). In the p38 and Akt-protein kinase gels, the cell extracts from the rat mesenteric arteries were applied to the gel as a positive control. For further details on the methodology, see Frodin et al. (2002).

2.2.1. Antibodies

Goat polyclonal antibody was used to detect ERK1/2 (Santa Cruz Biotechnology, USA), while a polyclonal rabbit antibody (Promega, USA) was used to detect phospho-ERK1/2 kinase. Rabbit polyclonal antibodies (Cell Signaling Technology, USA) were used to detect p38 MAP kinase, phospho-p38 MAP kinase (Thr180/Tyr182), Akt protein kinase, phospho-Akt protein kinase (Thr308) and phospho-Akt (Ser473). Peroxidase-conjugated anti-goat (DAKO, Denmark) and anti-rabbit (Amersham Pharmacia Biotech, UK) immunoglobulins were used as secondary antibodies. For development, we used SuperSignal West Dura Extended Duration Substrate (Pierce, Perbio, USA).

2.3. In vitro pharmacology

After incubation, the segments were immersed in temperature-controlled (37 °C) tissue baths containing a bicarbonate buffer solution (for composition, see below). The solution was continuously aerated with 5% CO_2 in O_2 resulting in a pH of 7.4. Each segment was mounted on two L-shaped prongs, one of which was attached to a Grass FT-03 transducer (Grass Instr., Quincy, USA) connected to a MacLab unit (ADInstruments, Hastings, UK) for continuous recording of isometric tension. A tension of 2 mN was applied to each segment and the segments were allowed to stabilise at this tension for 1 h before being exposed to a K^+-rich (63,5 mM) buffer solution with the same composition as the standard solution except that NaCl was replaced by an

equimolar concentration of KCl. The K^+-induced contraction was used as a reference for the contractile capacity, and the segment was used only if K^+ elicited reproducible responses over 0.5 mN. Concentration–response curves for the specific endothelin ET_B receptor agonist S6c were obtained by cumulative administration of the peptide. The vessels were then washed and allowed to recover for 1 h before ET-1 concentration–response curves were performed. For further methodological details, see Adner et al. (1996) and Uddman et al. (2002).

2.4. Buffer solutions and drugs

Standard buffer solution (mM): NaCl, 119; $NaHCO_3$, 15; KCl, 4.6; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $CaCl_2$, 1.5; glucose, 5.5. Analytical grade chemicals and double-distilled water were used for preparing all solutions. S6c and ET-1 (Auspeptide, Aus) were dissolved in water with bovine serum albumin (Kabi, Sweden) (0.1% w/v). All MAPK inhibitors were dissolved in DMSO and diluted in water. PD98059 was purchased from Sigma, St. Louis, USA. SB239063, SB408039, SB386023b and SB203580 were a generous gift from Dr. A.A. Parsons, GSK, UK.

2.5. Calculations and statistics

Data are expressed as mean values ± S.E.M. Contractile responses in each segment are expressed as a percentage of the K^+-induced contraction. E_{max} represents the maximal contraction induced by an agonist, and given either in absolute values or expressed as a percentage of the K^+-induced response for each vessel segment. The pEC_{50} value was calculated from the line between the concentrations above and below the midpoint of the concentration–response curve. Kruskal–Wallis nonparametric test with Dunn's post-hoc test was used for all statistical analyses. Differences were considered significant at $P < 0.05$.

2.6. Analysis of endothelin receptor mRNA

The vessels were snap frozen in −80 °C, and total cellular RNA was extracted using the FastRNA, Pro Green kit (Qbiogene) for 60 s in the FastPrep FP120 instrument (Qbiogene) following the suppliers' instructions. The resulting pellet was finally washed with 70% ethanol, air-dried and redissolved in 50 μl diethyl-pyrocarbonate (DEPC)-treated water. Reverse transcription of total RNA to cDNA was carried out using the GeneAmp RNA PCR kit (PE Applied Biosystems, Foster City, CA, USA) in a Perkin-Elmer DNA Thermal cycler. First strand cDNA was synthesized from 1 μg total RNA in a 20 μl reaction volume using random hexamers as primers. The reaction mixture was incubated at 25 °C for 10 min, 48 °C for 15 min, heated to 95 °C for 5 min and chilled to 5 °C for 5 min. Real-time PCR was performed in a GeneAmp 5700 Sequence Detection System using the GeneAmp SYBR® Green kit (Perkin-Elmer Applied Biosystems) with the cDNA synthesized above as template in a 50 μl reaction volume. A no template control was included in all experiments. The GeneAmp 5700 Sequence Detection System monitors the growth of DNA in real-time using an optics and imaging system, via the binding of a fluorescent dye to double-stranded DNA. Specific primers were designed as follows:

Endothelin ET_A receptor	forward	5′-GTCGAGAGGT GGCAAAGACC-3′
	reverse	5′-ACAGGGCGAA GATGACAACC-3′
Endothelin ET_B receptor	forward	5′-GATACGACAA CTTCCGCTCCA-3′
	reverse	5′-GTCCACGATGA GGACAATGAG-3′

Elongation factor-1 (EF-1) mRNA was used as a reference, since it is the product of a housekeeping gene, continuously expressed to a constant amount in cells. The EF-1 primers were designed as follows:

EF-1	forward	5′-GCAAGCCCATGTGTGTTGAA-3′
	reverse	5′-TGATGACACCCACAGCAACTG-3′

The real-time PCR was carried out with the following profile: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles with 95 °C for 15 s and 60 °C for 1 min. To prove that the cDNA of EF-1 and the ET receptors were amplified with the same efficacy during real-time PCR, standard curves were made where the C_T-values were plotted against cDNA concentration based on the equation:

$$C_T = \log\left(\frac{\text{concentration}}{(1+E)}\right)$$

where E is the amplification efficiency with the optimal value of 1. The amplification efficiency can be calculated from the slope of the standard curve with an ideal slope close to 3.3 (Fig. 1).

The amount of endothelin ET_B receptor mRNA was calculated as relative to the amount of EF-1 mRNA in the same sample by the formula:

$$X_0/R_0 = 2^{(\mathrm{CtR}-\mathrm{CtX})}$$

where X_0 = original amount of ET receptor mRNA, R_0 = original amount of EF-1 mRNA, CtR = C_T-value for EF-1 and CtX = C_T-value for the ET receptor. Statistical analyses

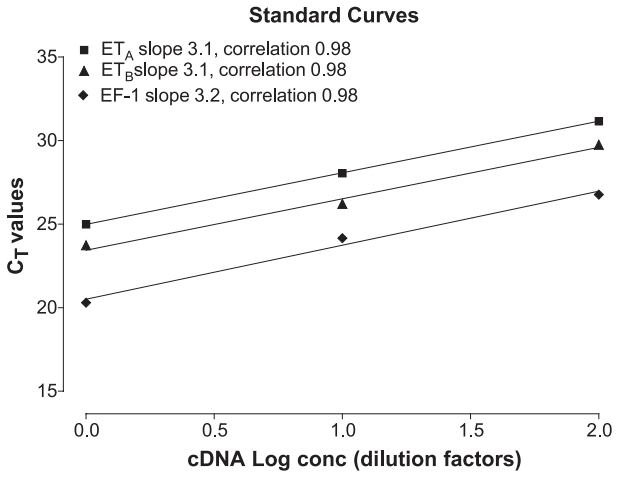

Fig. 1. Standard curves for each primer pair. *X*-axis = cDNA concentration (log dilution factor), *Y*-axis = C_T-values. EF-1: slope = 3.02; ET_A: slope = 3.01; ET_B: slope = 3.01.

were performed with Kruskal–Wallis nonparametric test with Dunn's post-hoc test, where $P < 0.05$ was considered significant.

3. Results

3.1. Western blot

In order to examine the presence of the different MAP kinase pathways and possible activation during organ culture, we used Western blot for studies of activation of ERK1/2, p38 and Akt-protein kinases in the cultured mesenteric arteries. Immunoblots with the MAP kinase-antibody detected bands corresponding to the 42- and 44-kDa isoforms of ERK1/2 MAPKs (Fig. 2A). The level of MAPK appears to be identical in fresh samples and in arteries incubated for 1, 2 and 3 h. In the fresh arteries, the signal for phospho-ERK1/2 kinase was barely visible. MAPK activation was observed in the cultured arteries and was most prominent after 3 h (Fig. 2B). Only the ERK1/2 isoforms of the MAPKs appeared to be phosphorylated as shown in Fig. 2B.

Immunoblots with the p38-antibody detected bands of approximately 43 kDa corresponding to the size of p38 in fresh arteries and in samples cultured for 1, 2 and 3 h (Fig. 2C). A band was also applied to the gel extract, which was applied to the gel as a positive control. Incubation of mesenteric arteries in culture medium for up to 3 h did not appear to activate p38, since there was no visible difference in the signals for phospho-p38 in the incubated samples as compared to fresh vessel segments (Fig. 2D). A clear signal was observed in the p38-activated cell extract, indicating successful immunoblotting.

Immunoblots with the Akt-protein kinase antibody detected bands at 60 kDa corresponding to the size of Akt-protein kinase in all the samples (Fig. 2E). After organ culture for 1 h, a signal for phospho-Akt-protein kinase was detected in the immunoblots. At 3 h of organ culture, the signal had faded (Fig. 2F).

3.2. Pharmacology

To study whether the ERK1/2 and p38 MAPK pathways are of importance in the up-regulation of the endothelin

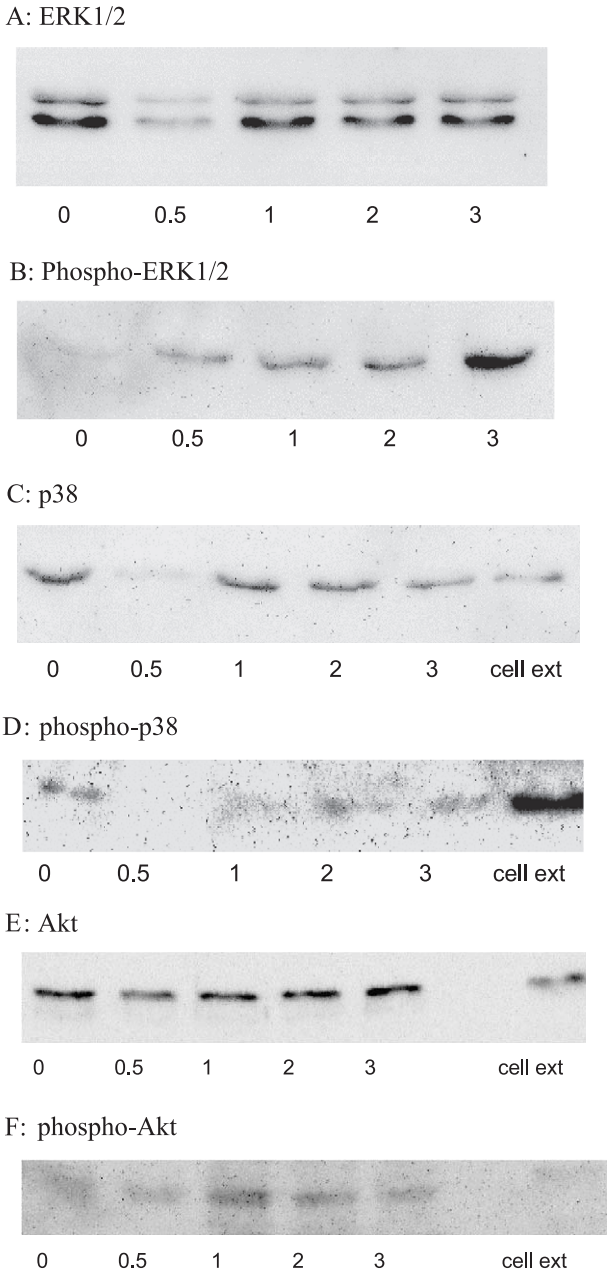

Fig. 2. Western blot of (A) ERK1/2 kinase, (B) phospho-ERK1/2 kinase, (C) p38 MAP kinase, (D) phospho-p38-MAP kinase, (E) akt-protein kinase and (F) phosphor-akt-protein kinase following organ culture for 1 to 3 h.

Table 1
Sarafotoxin 6c and endothelin-1 induced contractions in mesenteric arteries incubated for 24 h with different MAPK kinase inhibitors

	N	K^+ mean ± S.E.M.	$S6c_{max}$ ± S.E.M.	$S6c_{max}$ (% of K^+) ± S.E.M.	S6c pEC_{50} ± S.E.M.	$ET\text{-}1_{max}$ ± S.E.M.	$ET\text{-}1_{max}$ (% of K^+) ± S.E.M.	ET-1 pEC_{50} ± S.E.M.
Control	10	3.18 ± 0.35	3.00 ± 0.66	120 ± 30	8.46 ± 0.12	7.02 ± 0.80	228 ± 26	8.01 ± 0.11
PD98059	9	3.95 ± 0.36	2.67 ± 0.39	70 ± 11	8.54 ± 0.11	6.98 ± 0.68	177 ± 8	8.02 ± 0.13
SB408039	9	2.23 ± 0.46	0.33 ± 0.08[b]	20 ± 5[a]	9.36 ± 0.25[b]	4.56 ± 1.04	230 ± 40	8.04 ± 0.26
SB239063	9	2.64 ± 0.39	1.34 ± 0.24	53 ± 7	8.66 ± 0.15	7.78 ± 0.59	321 ± 28	8.00 ± 0.13
SB230580	8	2.63 ± 0.49	1.96 ± 0.42	100 ± 23	8.59 ± 0.18	7.73 ± 1.27	370 ± 102	8.08 ± 0.13
SB386023[b]	5	1.56 ± 0.55	0.70 ± 0.72[c]	25 ± 21[b]	9.00 ± 0.78	7.56 ± 0.57	668 ± 192[a]	7.87 ± 0.13

[a, b] and [c] denotes statistical significance [$p < 0.05$], [$p < 0.01$] and [$p < 0.001$] vs. control.

receptors, segments of rat mesenteric arteries were cultured for 24 h with the different MAPK inhibitors (PD98059, SB239063, SB203580, SB408039 and SB386023b). The contractile responses to the endothelin ET_B receptor agonist S6c and ET-1 (after endothelin ET_B receptor desensitisation) were investigated.

In control vessels, S6c induced a concentration-dependent contraction of 120 ± 30% of the potassium induced

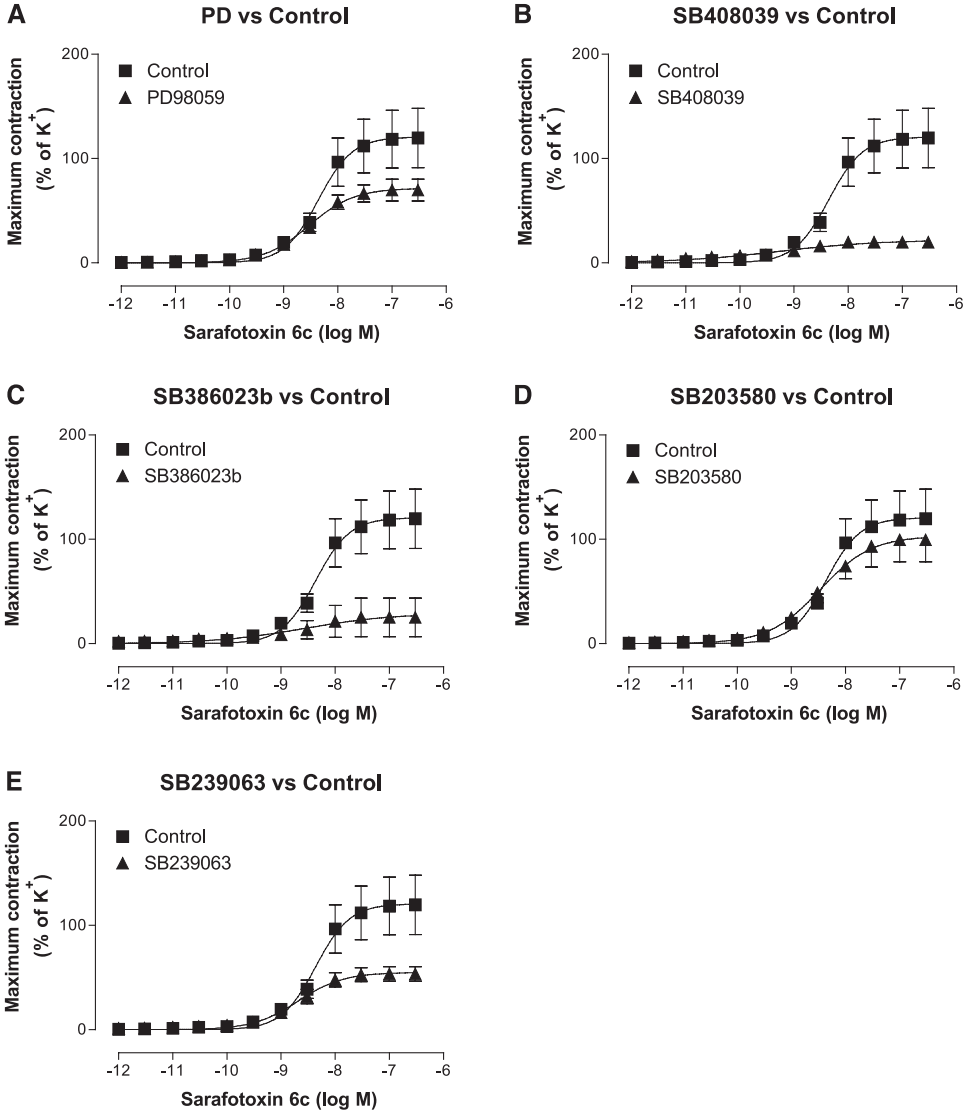

Fig. 3. Concentration response curves for S6c in the presence of the inhibitors: (A) PD98059, (B) SB408039, (C) SB386023b, (D) SB203580 and (E) SB239063. Data are shown as mean ± S.E.M.

responses. Co-culture with the MAPK inhibitor SB408039 attenuated the S6c induced response, now only reaching 20 ± 5%. SB386023b also attenuated the responses to endothelin ET_B receptor activation with an E_{max} of 25 ± 21%. Following co-culture with SB239063, the S6c induced responses reached 53 ± 7% (nonsignificant). Neither PD98059 nor SB203580 affected the S6c induced responses (Table 1, Fig. 3).

The ET-1 induced endothelin ET_A receptor contractions after endothelin ET_B receptor desensitisation was increased following organ culture with SB386023b. This could be an artefact due to the fact that this inhibitor reduced potassium induced responses. The increase was not significant when mN values were compared. None of the other MAPK inhibitors tested affected the ET-1 induced responses (Table 1, Fig. 4)

3.3. Real-time PCR

To confirm that the alterations in S6c and ET-1 induced responses were due to changes in receptor expression, we studied changes in endothelin ET_B receptor mRNA levels with real-time PCR following organ culture with or without the above MAPK inhibitors. Organ culture with the MAPK inhibitors SB408039 and SB386023b resulted in reduced levels of the endothelin ET_B receptor mRNA expression as compared to control (Fig. 5A). None of the MAPK inhibitors used in this

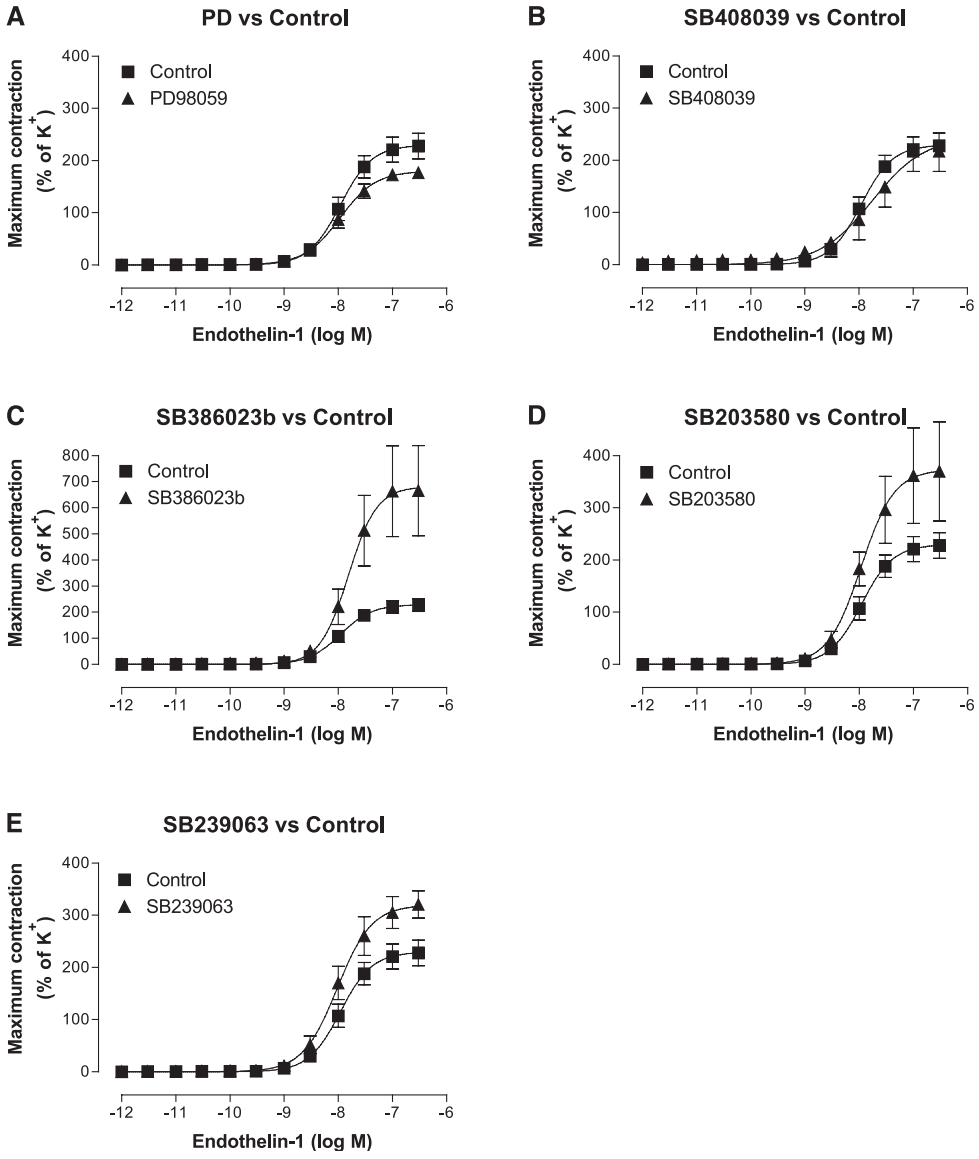

Fig. 4. Concentration response curves for ET-1 in the presence of the MAPK inhibitors; (A) PD98059, (B) SB408039, (C) SB386023b, (D) SB203580 and (E) SB239063. Data are shown as mean ± S.E.M.

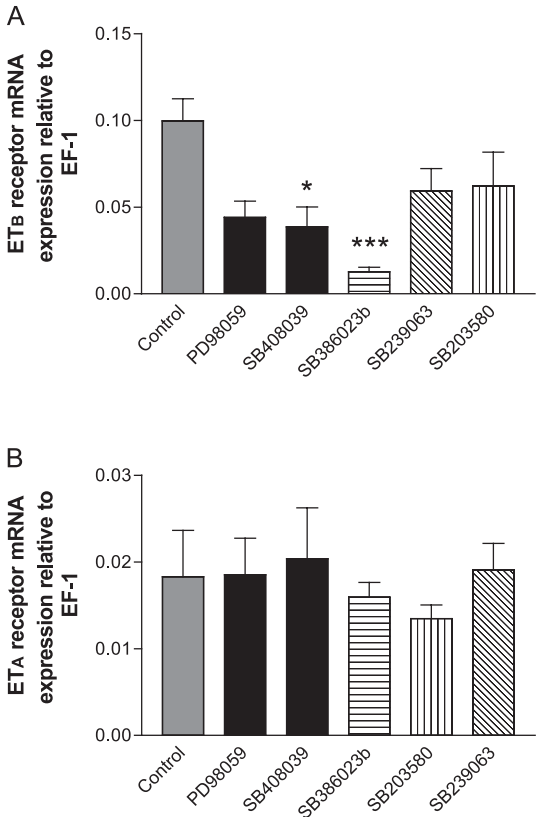

Fig. 5. (A) Relative endothelin ET_B receptor and (B) endothelin ET_A receptor mRNA levels in the fresh rat mesenteric artery and following organ culture with PD98059, SB239063, SB203580, SB408039 and SB386023b. Data are presented as mean ± S.E.M relative to EF-1, $n = 5–6$. (* and *** denotes statistical significance [$p < 0.05$] and [$p < 0.001$] vs. the cultured control).

study affected the endothelin ET_A receptor mRNA expression (Fig. 5B).

4. Discussion

Previous studies have shown up-regulation of ET receptors in experimental focal ischemia (Stenman et al., 2002), cerebrovascular disease (Hansen-Schwartz et al., 2002b) and in coronary artery infarction in man (Wackenfors et al., unpublished results). The organ culture model provides a convenient method to study the time course of both mRNA and functional endothelin receptor changes (Möller et al., 2002). There are regional variations in various vascular beds (Adner et al., 1998b) and cytokines can enhance this response (Uddman et al., 1999). The receptor increase occurs via de novo transcription with subsequent translation (Möller et al., 1997).

In the present study, we have examined (i) which type of MAPK activation that occurs during organ culture by analysing phosphorylated and non-phosphorylated MAP kinases using Western blot, and (ii) the inhibition of endothelin ET_B receptor up-regulation at both mRNA and functional levels by various types of MAPK inhibitors. With the Western blotting, we observed the presence of the MAPKs ERK1/2 and p38. Only the ERK1/2 was present in its phosphorylated form, reaching the highest level at 3 h following organ culture. Thus, there was a time-dependent activation of the ERK1/2 kinase pathway but no activation of p38 or Akt-kinase after 3 h of culture. It is therefore reasonable to suggest that the cell uses the ERK1/2 pathway to initiate endothelin ET_B receptor up-regulation during organ culture.

In order to further understand the role of the MAPKs in the receptor modulation pathway, we aimed to couple the activation of MAPKs to endothelin receptor up-regulation. The effect of the different MAPK inhibitors on the endothelin ET_B mRNA expression, an event preceding the functional receptor responses, was studied (Möller et al., 1997, 2002). Of the MAPK inhibitors tested in this study PD98059 and SB408039 both affected the ERK1/2 system. SB408039 blocks the enzymatic activity of MEK1/2, the kinases of ERK1/2 (Favata et al., 1998), whereas PD98059 blocks phosphorylation of MEK1b (Alessi et al., 1995). Of these, the first substance had a good inhibitory effect on the up-regulation of the endothelin ET_B receptor. PD98059 did not have an equally strong effect. It is not inconceivable that when the substance is added MAPK phosphorylation is already initiated or that the substance is less efficacious as a blocker of the MAPK pathway. The substances SB203580 and SB239063 are both selective inhibitors of the p38 system. SB203580 had no effect on the endothelin ET_B receptor mediated responses while a weak, nonsignificant, reduction of the S6c induced response was noted following incubation with SB239063.

SB386023b is an inhibitor of raf, an upstream enzyme involved in the activation of MAPK, primarily of the ERK1/2 system (Yue et al., 2000). In our study, SB386023b had a strong inhibitory effect on endothelin ET_B receptor up-regulation both functionally, as shown by in vitro endothelin ET_B receptor responses and on the transcriptional level by real-time PCR. The endothelin ET_A receptor responses seemed to be increased when compared with the potassium induced responses; however, the results where unaffected when measured as mN values. Thus, the increased endothelin ET_A receptor response is probably illusory and due to the reduction in potassium induced responses. The latter observation clearly requires future evaluation.

Real-time PCR demonstrated an up-regulation of endothelin ET_B receptor mRNA in incubated control vessels compared to fresh vessels (not shown). There was no effect on the endothelin ET_A receptor mRNA confirming previous findings (Möller et al., 1997). Following incubation with MAPK inhibitors, the vessel segments showed reduced endothelin ET_B receptor mRNA levels after culture with SB386023b and SB408039, both affecting the ERK1/2 pathway. None of the other MAPK inhibitors achieved a

significant decrease in endothelin ET_B receptor mRNA levels after organ culture.

In previous studies, we have shown that the endothelin ET_B receptor is up-regulated following organ culture (Adner et al., 1996, 1998b) and that this up-regulation involves the protein kinase C pathway (Hansen-Schwartz et al., 2002a; Henriksson et al., 2003; Uddman et al., 2002). Here, we have shown that the MAPKs are important mediators in regulating endothelin ET_B receptor transcription, the strongest effect was seen for SB386023b which is a raf inhibitor, acting upstream from the MEK/ERK1/2 cascade, and SB408039 which is a specific MEK 1/2 inhibitor. Our data support the hypothesis that MAPKs, particularly of the MEK/ERK1/2 pathway, are involved in up-regulation of endothelin ET_B receptors. The MAP kinase pathways are thought to act downstream from protein kinase C in the smooth muscle cell regulatory cascade (Schonwasser et al., 1998). Thus, it is reasonable to speculate that endothelin ET_B receptor up-regulation is initiated by a pathway from PKC, via raf and ERK1/2.

We did not observe any effect of MAPK inhibition on endothelin ET_A receptor expression or on ET-1 induced responses. In this model, the ET-1 responses can be considered to be primarily an endothelin ET_A receptor response as the endothelin ET_B receptor is desensitised by the prior application of S6c (Adner et al., 2001; Lodge et al., 1995). The lack of MAPK inhibitor effect is possibly due to the fact that the endothelin ET_A receptor is not affected by organ culture in this artery (Adner et al., 1998b).

In conclusion, we have revealed that the ERK1/2 pathway is activated in rat mesenteric arteries during organ culture. Endothelin ET_B receptor up-regulation may be an important pathophysiological event that occurs in cardiovascular diseases. Inhibition of MEK 1/2 or of raf blocks both transcription and the subsequent up-regulation of the endothelin ET_B receptor in the mesenteric artery.

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