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RESEARCH PAPER

Effect of inhibition of extracellular signal-regulated kinase on relaxations to β-adrenoceptor agonists in porcine isolated blood vessels

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Background and purpose: Stimulation of vascular β-adrenoceptors causes vasodilatation through activation of adenylyl cyclase (AC) and plasma membrane potassium channels, and β-adrenoceptors have been linked to activation of extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase in various cell lines. However, how these findings relate to functional responses in intact tissues is largely unknown. The aim of this study, therefore, was to investigate the role of ERK in β-adrenoceptor-induced vasodilatation.
Experimental approach: Segments of porcine coronary artery were mounted in a Mulvany wire myograph and bathed in Krebs–Henseleit buffer gassed with 95% O_2/5% CO_2 and maintained at 37°C. Tissues were pre-contracted with the thromboxane mimetic U46619, endothelin-1 or KCl. Cumulative concentration–response curves to β-adrenoceptor agonists or forskolin were then carried out in the absence or presence of the mitogen-activated protein kinase kinase (MEK) inhibitors PD98059 (10 or 50 μM) or U0126 (10 μM).
Key results: PD98059 caused a concentration-dependent leftward shift in response to isoprenaline (pEC_{50} control, 7.5 ± 0.1; 50 μM PD98059, 8.1 ± 0.1: $P < 0.05$). Inhibition of MEK also enhanced the maximum relaxation seen with salbutamol, but not the responses to the β_1-adrenoceptor selective agonist xamoterol or the AC activator forskolin. There was no enhancement of the relaxations to β-adrenoceptor agonists after inhibition of ERK activation in tissues pre-contracted with KCl or treated with the K^+ channel blocker tetraethylammonium.
Conclusions and implications: These data indicate that ERK inhibits β_2-adrenoceptor-mediated vasodilatation through a mechanism which may involve inactivation of plasma membrane potassium channels.
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Keywords: ERK; β-adrenoceptors; smooth muscle; vasodilatation

Abbreviations: PCA, porcine coronary artery

Introduction

β-Adrenoceptors are exploited as therapeutic targets in the treatment of a range of diseases, particularly those involving the respiratory and cardiovascular systems, where airway β_2-adrenoceptors and myocardial β_1-adrenoceptors, respectively, are selectively targeted. Stimulation of vascular β-adrenoceptors elicits a relaxation of vascular smooth muscle cells, causing vasodilatation. This process is of particular relevance to conditions such as hypertension, which may be associated with elevated vascular tone, and myocardial ischaemia, during which perfusion of the myocardium is inadequate.

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β-Adrenoceptor-mediated vasodilatation appears to involve a number of components. Possibly the most investigated is the adenylyl cyclase–cyclic adenosine monophosphate (AC–cAMP) signalling pathway, which activates cAMP-dependent protein kinase (PKA), reducing the calcium sensitivity of contractile proteins (Murray, 1990). The gaseous radical nitric oxide (NO) has been identified as another likely mediator, because both endothelium denudation and NO synthase inhibition reduce β-adrenoceptor-mediated relaxation responses in a variety of vessels (Graves and Poston, 1993; Cardillo *et al.*, 1997). β–Adrenoceptor agonists may also relax vascular smooth muscle by activating a range of potassium channels, including ATP-sensitive K^+ channels (K_{ATP}; Randall and McCulloch, 1995) and large conductance Ca^{2+}-activated K^+ channels (BK_{Ca}; White *et al.*, 2001).

The extracellular signal-regulated kinases (ERKs) are one of the three main families of mitogen-activated protein kinases. Aside from their roles as important mediators of cell growth

and differentiation, they are also known to regulate contractile responses in vascular tissues (Roberts, 2001). A possible role for the ERKs in smooth muscle relaxation is suggested by previous experiments in which isoprenaline and other β-adrenoceptor ligands increase ERK activation in cultured cells expressing β-adrenoceptors (Friedman *et al.*, 2002; Baker *et al.*, 2003). However, to our knowledge, no previous studies have shown how these cell-based findings relate to functional responses in intact tissues. The aim of this study, therefore, was to investigate the role of ERK in β-adrenoceptor-induced vasodilatation.

Methods

Tissue preparation

Hearts and trotters from freshly slaughtered pigs were transported from a local abattoir to the laboratory in ice-cold Krebs–Henseleit buffer (see below). From each heart, the anterior descending coronary artery was dissected and stripped of all adipose and connective tissue, and palmar lateral veins were removed from the trotters. The dissected blood vessels were then refrigerated overnight at 4°C in Krebs–Henseleit solution containing 2% Ficoll, and pre-gassed with O_2–CO_2 mixture (95:5). Krebs–Henseleit solution was composed, in mM, of the following: NaCl, 128; KCl, 4.8; $MgSO_4$, 1.1; $NaHCO_3$, 25; KH_2PO_4, 1.2; D-glucose, 12; $CaCl_2$, 1.25.

The following day, 2 mm ring segments were cut, without removing the endothelium, from the palmar lateral vein or the distal section of the coronary arteries and set up in a Mulvany, four-channel wire myograph attached to a Macintosh computer via a MacLab data acquisition system (ADInstruments Ltd, Charlsgrove, UK). The 5 mL baths contained Krebs–Henseleit solution gassed with 95% O_2, 5% CO_2 maintained at 37°C. A tension of 5 g (coronary artery) or 2 g (palmar lateral vein) was applied to each ring segment following a 20 min equilibration period.

Experimental procedure

The tissues were exposed three times to 60 mM KCl to determine their maximal contractile capacities, with thorough rinsing and a 20-min recovery period following each KCl challenge. The tissues were then incubated in Krebs–Henseleit buffer for 45 min in either the absence or presence of the selective mitogen-activated protein kinase kinase (MEK) inhibitor PD98059 (10 or 50 μM) in order to inhibit ERK activation. Control tissues received vehicle only [0.26% v/v dimethyl sulphoxide (DMSO) for 50 μM PD98059 and 0.052% v/v DMSO for 10 μM PD98059]. Finally, the tissues were pre-contracted to approximately 65–80% of the maximal KCl contractile response using the thromboxane mimetic U46619 (concentration range 10–20 nM), before relaxations were induced using cumulative concentrations of isoprenaline (1 nM–10 μM), salbutamol (10 nM–30 μM), xamoterol (10 nM–30 μM) or forskolin (1 nM–10 μM). In a separate set of experiments, the effect of PD98059 on salbutamol relaxations in the presence of 10 nM CGP 20712 (a β_1-adrenoceptor-selective antagonist) was determined. The experiments were repeated using the structurally dissimilar MEK inhibitor U0126 (10 μM), as well as its inactive congener U0124 (10 μM), instead of PD98059. In other studies, tissues were pre-contracted with endothelin-1 (ET-1) or KCl to 65–80% of the 60 mM KCl response. The level of pre-contraction was the same in all tissues.

In separate experiments, tissue segments were exposed to the combination of PD98059 (50 μM) and the non-selective potassium channel inhibitor tetraethylammonium (TEA; 10 mM) for 45 min; control segments received vehicle only (0.26% DMSO). In order to determine the effect of inhibition of ERK activation on the pre-contractile agents, tissues were exposed to 50 μM PD98059 for 1 h prior to concentration–response curves to U46619 (1 nM–3 μM) or ET-1 (0.1–30 nM). The β_1-adrenoceptor-selective antagonist CGP 20712 (10 nM) was used to determine the role of β_1-adrenoceptors in salbutamol- and xamoterol-induced relaxations.

Statistical analyses

The computer program Prism (GraphPad Software, Inc., La Jolla, CA, USA) was used to analyse the data. Results are expressed as means ± SEM. Comparisons between groups were made using the Student's two-tailed, unpaired *t*-test with $n \geq 5$. A P value < 0.05 was considered statistically significant.

Materials

(5Z, 9α, 11α, 13E, 15(*S*))-15-hydroxy-9 (11) methanoepoxy-prosta-5,13-dien-1 oic acid (U46619) (Axxora, Bingham, Nottinghamshire); 2-amino-3-methoxyflavone (PD98059) (Calbiochem, Beeston, Nottingham); 1,4-diamino-2,3-dicyano-1,4-*bis* (2-aminophenylthio) butadiene (U0126), *bis* [amino (methylthio) methylene] butanedinitrile (U0124); 1-[2-((3-carbamoyl-4-hydroxy)phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl) phenoxy]-2-propanol dihydrochloride (CGP20712A) (Tocris Bioscience, Bristol, UK); forskolin (Axxora); salbutamol (Axxora), xamoterol (Sigma, Poole, Dorset, UK). The remaining chemicals were obtained from Sigma.

Nomenclature

Drug and molecular target nomenclature conforms to the British Journal of Pharmacology's *Guide to Receptors and Channels* (Alexander *et al.*, 2008).

Results

The effect of MEK inhibition on β-adrenoceptor-mediated relaxation

Isoprenaline caused a concentration-dependent relaxation of segments of porcine distal coronary artery (PCA) pre-contracted with U46619 (Figure 1A). Pre-incubation with 10 μM PD98059 caused a twofold leftward shift in the concentration–response curve to isoprenaline (Figure 1A), with the pEC_{50} values increasing from 7.9 ± 0.1 in controls (0.05% DMSO) to 8.1 ± 0.1 in PD98059-treated vessels ($P < 0.05$, $n = 7$). A fivefold enhancement in the response was seen after pre-incubation with 50 μM PD98059 (Figure 1B), with pEC_{50} values increasing from 7.5 ± 0.1 in controls (0.26%

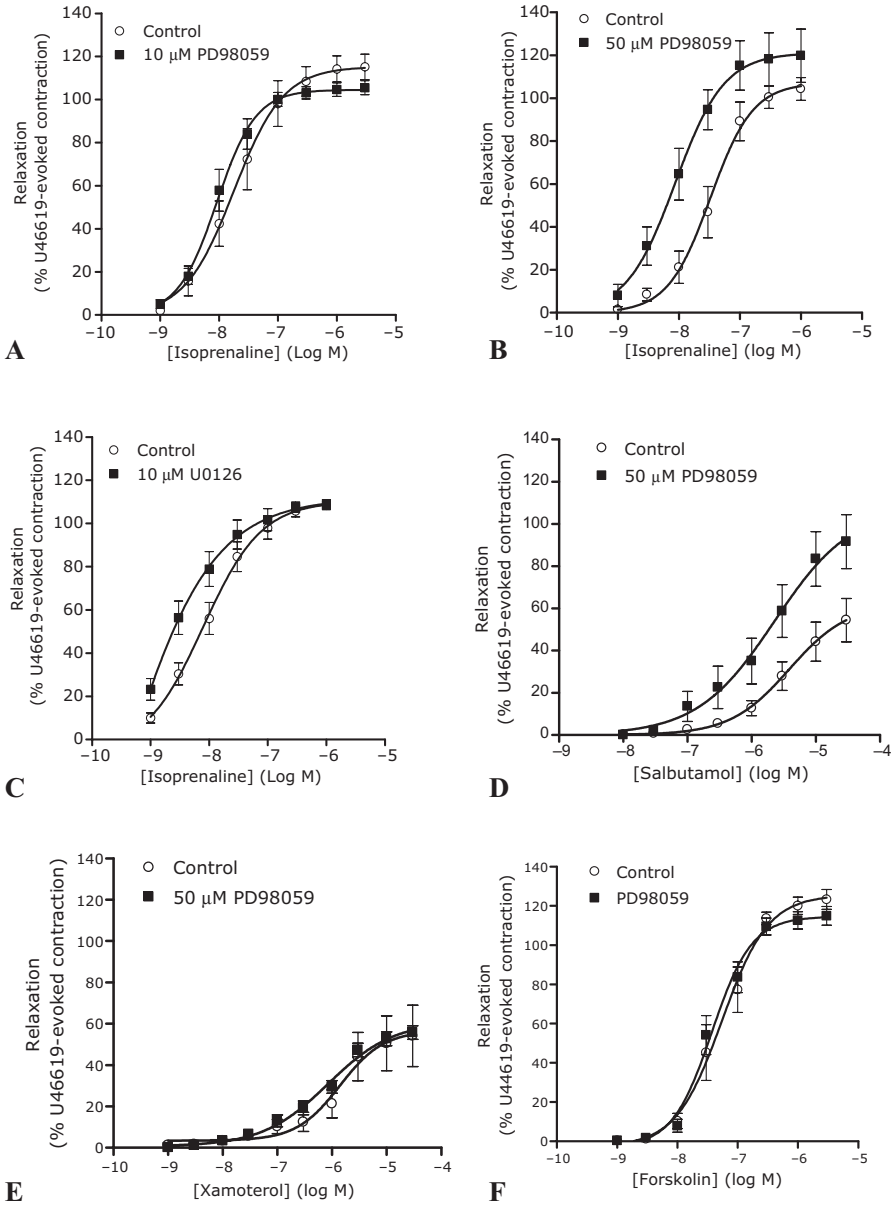

Figure 1 (A) Log concentration–response curves to isoprenaline in porcine coronary artery (PCA) segments. Relaxation responses, shown as means ± SEM ($n = 7$), are expressed as a percentage of the U46619-evoked contraction, and were carried out in either the absence or presence of PD98059 (10 μM). (B) Log concentration–response curves to isoprenaline in PCA segments. Relaxation responses, shown as means ± SEM ($n = 6$), are expressed as a percentage of the U46619-evoked contraction, and were carried out in either the absence or presence of PD98059 (50 μM). (C) Log concentration–response curves to isoprenaline in PCA segments. Relaxation responses, shown as means ± SEM ($n = 15$), are expressed as a percentage of the U46619-evoked contraction, and were carried out either with or without U0126 (10 μM). (D) Log concentration–response curves to salbutamol in PCA segments. Relaxation responses, shown as means ± SEM ($n = 9$), are expressed as a percentage of the U46619-evoked contraction, and were carried out either with or without PD98059 (50 μM). (E) Log concentration–response curves to xamoterol in PCA segments. Relaxation responses, shown as means ± SEM ($n = 7$), are expressed as a percentage of the U46619-evoked contraction, and were carried out either with or without PD98059 (50 μM). (F) Log concentration–response curves to forskolin in PCA segments. Relaxation responses, shown as means ± SEM ($n = 6$), are expressed as a percentage of the U46619-evoked contraction, and were carried out either with or without PD98059 (50 μM).

DMSO) to 8.1 ± 0.1 in PD98059-treated vessels ($P < 0.05$). There was no significant effect on the maximum response. Similar effects were seen with the structurally dissimilar MEK inhibitor U1026. Pre-incubation with 10 μM U0126 also caused a leftward shift in the concentration–response curve to isoprenaline (Figure 1C; $pEC_{50} = 8.0 \pm 0.1$ in controls compared to 8.4 ± 0.2 in U0126-treated vessels; $P < 0.05$, $n = 15$). U0124 (10 μM), which is structurally similar to U0126, but does not inhibit MEK, did not cause a shift in the isoprenaline concentration–response curves (pEC_{50} 7.9 ± 0.1 in controls vs. 7.9 ± 0.1 in the presence of U0124, $n = 4/5$). Vessels were pre-contracted such that there was no significant difference in tone between control vessels and PD98059-exposed vessels (70 ± 5% and 69 ± 10% of maximal KCl response respectively).

Table 1 Relaxation responses to isoprenaline, salbutamol and forskolin in segments of porcine palmar lateral vein in the absence (control) or presence of 50 μM PD98059

Agonist		*Max*	*pEC_{50}*
Isoprenaline	Control	96 ± 3% (n = 8)	7.5 ± 0.2 (n = 8)
	+ PD98059	103 ± 1% (n = 8)*	8.2 ± 0.1 (n = 8)**
Salbutamol	Control	71 ± 10% (n = 4)	6.6 ± 0.1 (n = 4)
	+ PD98059	94 + 2% (n = 4)*	7.2 ± 0.1 (n = 4)**
Forskolin	Control	100 ± 2% (n = 4)	6.6 ± 0.1 (n = 4)
	+ PD98059	103 ± 4% (n = 4)	6.6 ± 0.2 (n = 4)

*P < 0.05 versus control.
**P < 0.01 versus control.
Control tissues were exposed to 0.26% DMSO. Shown are the maximum percentage relaxation and the pEC_{50} values expressed as means ± SEM.

In order to determine the subtype specificity of this effect, relaxations of PCA segments were carried out with selective β-adrenoceptor agonists. Pre-incubation with 50 μM PD98059 caused a significant enhancement of the relaxations induced by the β_2-adrenoceptor partial agonist salbutamol (Figure 1D). Neither of the curves reached a maximum response. Therefore, R_{Max} and EC_{50} values could not be calculated. However, it is clear that the responses to salbutamol were enhanced in the presence of PD98059; the response to 10 μM salbutamol was increased from 44 ± 9% in control tissues to 83 ± 13% relaxation in tissues incubated with 50 μM PD98059 (P < 0.05). In contrast, responses to the β_1-adrenoceptor selective agonist, xamoterol, were unaltered in the presence of 50 μM PD98059 (Figure 1E). In a separate set of experiments, we demonstrated that the xamoterol relaxations were inhibited by the β_1-adrenoceptor-selective antagonist CGP20712A (10 nM) (pEC_{50} 7.2 ± 0.3 in controls compared to 5.6 ± 0.3 with CGP20712A; P < 0.01, n = 4/5). CGP20712A had no significant effect on the salbutamol relaxations, although there was a trend for a slight impairment at the higher concentrations of salbutamol (data not shown), indicating that salbutamol activates β_1-adrenoceptors at higher concentrations. Therefore, in order to clarify whether the enhanced relaxations to salbutamol were due to an effect through β_2-adrenoceptors, we determined the effect of 50 μM PD98059 on salbutamol relaxations in the presence of 10 nM CGP 20712A. Under these conditions, PD98059 still enhanced the salbutamol-induced relaxation (the response to 10 μM salbutamol was increased from 74 ± 6% in control tissues to 97 ± 3% relaxation in tissues incubated with 50 μM PD98059 (P < 0.01, n = 8/12).

To identify whether these effects were replicated in other vascular tissues, we investigated β-adrenoceptor relaxations in the porcine palmar lateral vein. As in the coronary artery, the presence of 50 μM PD98059 evoked a leftward shift in concentration–response curves to isoprenaline and a slight increase in the maximum response (Table 1). Similar enhancements were observed with salbutamol, which produced maximal responses in this tissue (Table 1). We were unable to detect a relaxation through β_1-adrenoceptors in this tissue (data not shown).

The effect of MEK inhibition on pre-contracting agent

In this series of experiments, cumulative concentration–response curves of U46619-evoked contractions of vessel segments exposed to either PD98059 (50 μM) or vehicle (DMSO) were constructed. PD98059 inhibited U46619-evoked contractions of PCA, shifting the concentration–response curve to the right (control pEC_{50} = 7.6 ± 0.1, PD98059 pEC_{50} = 7.3 ± 0.1; P < 0.01; Figure 2). There was no significant effect on the maximum response. On the other hand, in the palmar lateral vein, 50 μM PD98059 did not affect U46619-evoked contractions (maximum responses in controls and PD98059-treated vessels: 131 ± 24% and 151 ± 5%, respectively; pEC_{50} in controls and PD98059-treated vessels: 8.8 ± 0.2 and 8.6 ± 0.1, respectively; n = 5). Furthermore, pre-incubation with 50 μM PD98059 also had no effect on the ET-1-induced contraction in the PCA (maximum responses in controls and PD98059-treated vessels: 78 ± 12% and 74 ± 16%, respectively; pEC_{50} in controls and PD98059-treated vessels: 8.2 ± 0.1 and 8.1 ± 0.2, respectively; n = 6).

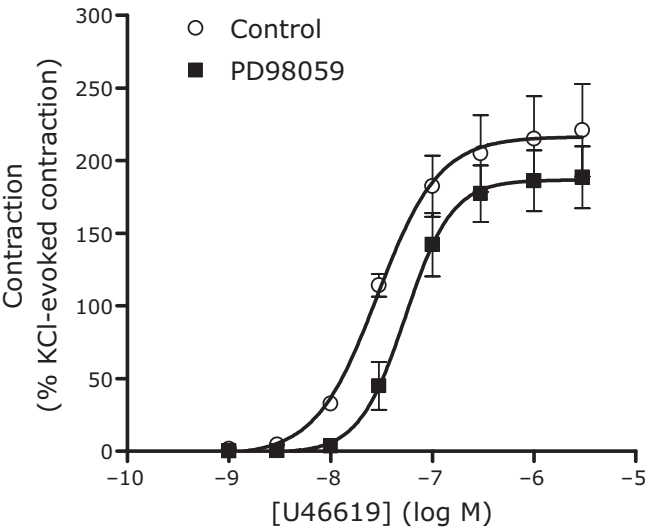

Figure 2 Log concentration–response curves to U46619 in porcine coronary artery segments. Contractile responses, shown as means ± SEM (n = 5), are expressed as a percentage of the KCl-evoked contraction, and were carried out either with or without PD98059 (50 μM).

The effect of MEK inhibition on forskolin-induced relaxation

The AC activator forskolin elicited a concentration-dependent relaxation of PCA segments (Figure 1F). Responses to forskolin were unaffected by MEK inhibition with 50 μM PD98059.

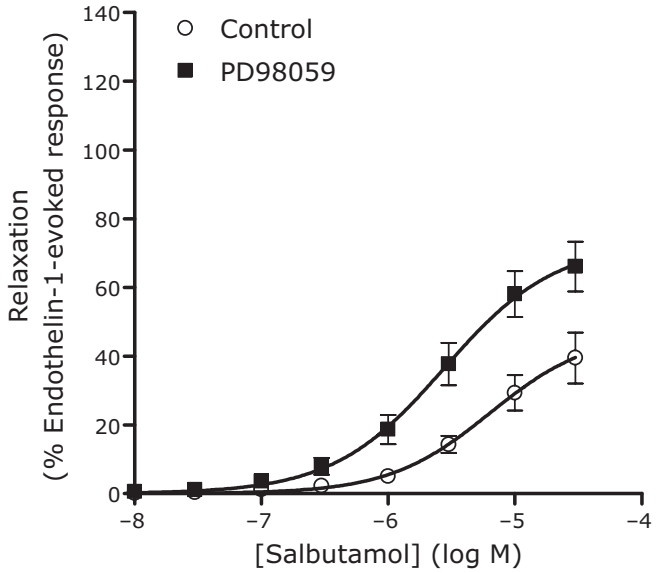

Figure 3 Log concentration–response curves to salbutamol in porcine coronary artery segments. Relaxation responses, shown as means ± SEM (n = 16/17), were expressed as a percentage of the endothelin-1-evoked contraction, and are carried out either with or without PD98059 (50 μM).

These findings are consistent with results obtained from similar experiments carried out on palmar lateral vein segments (Table 1).

The effect of different pre-contracting agent on the relaxation response

In order to determine the role of the pre-contracting agent in the effect of inhibition of ERK activation on the relaxation to β-adrenoceptor agonists, PCA segments were relaxed with cumulative concentrations of salbutamol following pre-contraction with ET-1 (Figure 3). Relaxations to salbutamol were lower in tissues pre-contracted with ET-1 compared to tissues pre-contracted with U46619. However, pre-incubation of the vessels with 50 μM PD98059 enhanced relaxations to salbutamol in these tissues, with an increase in the maximum response from 40 ± 7% to 66 ± 7% relaxation ($P < 0.05$, n = 16/17). The pre-contraction was carried out such that there was no significant difference between the level of evoked tone in PD98059-treated vessels and control vessels (85 ± 7% and 83 ± 9% of maximal KCl response respectively).

Role of K^+ channels

In order to determine whether the enhancement of the β-adrenoceptor relaxation is through an effect on K^+ channels, segments of PCA were pre-contracted with KCl (to 65–80% of the 60 mM KCl response). Under these conditions, the relaxations to β-adrenoceptor agonists were resistant to MEK inhibition (Figure 4A). In comparison, segments of PCA were pre-contracted with U46619 in the presence or absence of the K^+ channel blocker TEA (10 mM). Again, under these conditions, inhibition of ERK activation with PD98059 failed to enhance the relaxation to β-adrenoceptor agonist (Figure 4B).

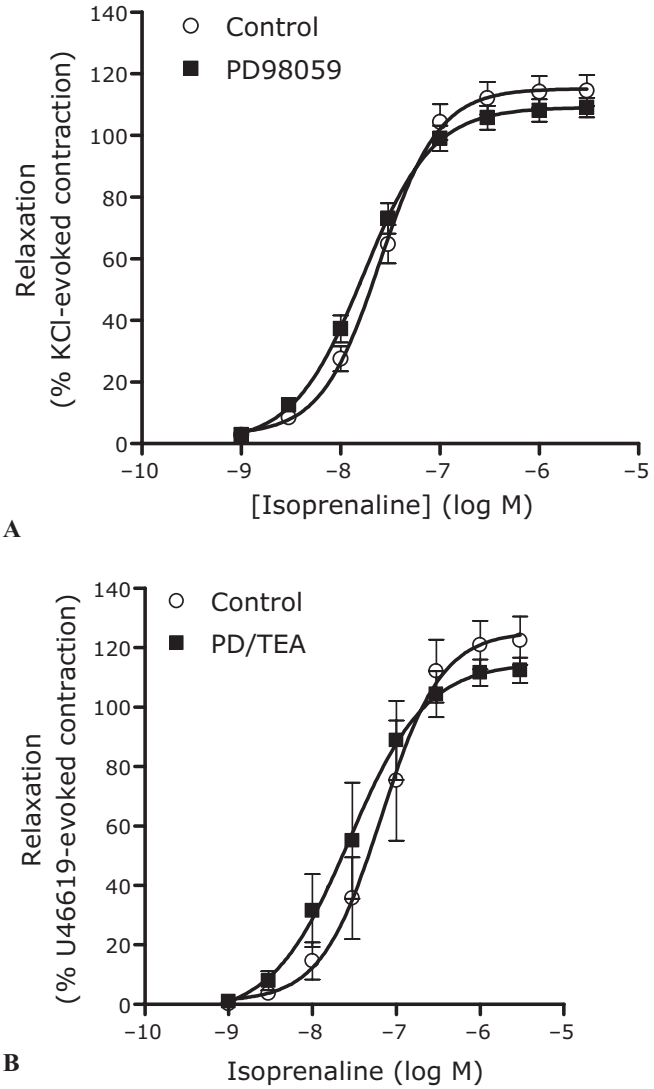

Figure 4 (A) Log concentration–response curves to isoprenaline in porcine coronary artery (PCA) segments after pre-contraction with KCL. Relaxation responses, shown as means ± SEM (n = 5), are expressed as a percentage of the KCl-evoked contraction, and were carried out either with or without PD98059 (50 μM). (B) Log concentration–response curves to isoprenaline in PCA segments. Relaxation responses, shown as means ± SEM (n = 6), are expressed as a percentage of the U46619-evoked contraction, and were carried out either with or without PD98059 (50 μM) and tetraethylammonium (10 mM).

Discussion

This study demonstrates that inhibition of ERK activation with the selective MEK inhibitors PD98059 (Alessi *et al.*, 1995; Davies *et al.*, 2000) and U0126 (Alessi *et al.*, 1995; Favata *et al.*, 1998) enhanced the relaxation to the β-adrenoceptor agonist isoprenaline in the PCA and the porcine palmar lateral vein. Our evidence suggests that this response was selective for the β_2-adrenoceptor subtype in that salbutamol-induced relaxations were also enhanced by inhibition of ERK activation, whereas relaxations caused by the β_1-adrenoceptor-selective agonist xamoterol were unaffected by inhibition of ERK activation. Furthermore, inhibition of ERK activation enhanced

the salbutamol-induced relaxations in the presence of the β_1-adrenoceptor-selective antagonist CGP20712A, thus demonstrating the β_1-adrenoceptors were not involved.

The mechanism underlying this enhancement of the relaxation to β-adrenoceptor agonists is unclear at present. Previous studies have demonstrated that ERK is involved in the contractile response to a number of different agonists (Florian and Watts, 1998; Roberts, 2001). So, it is possible that the enhancement of the relaxation to β-adrenoceptor agonists is due to inhibition of the pre-contraction, rather than enhancement of the response to β-adrenoceptors *per se*. However, there are a number of arguments against this as a mechanism. Firstly, relaxations to the AC activator forskolin and the β_1-adrenoceptor-agonist xamoterol were unaffected by inhibition of ERK activation. If the enhancement of the relaxation were due to inhibition of the pre-contraction, then all relaxation responses should be similarly enhanced. Secondly, although inhibition of ERK activation in the PCA caused inhibition of the U46619-induced contraction, there was no effect on the ET-1-induced contraction in this tissue. Furthermore, the U46619-induced contraction in the porcine palmar lateral vein was unaffected by inhibition of ERK activation (also suggesting that there are differences in the role of ERK activation in U46619-induced contractions between blood vessels). Therefore, the enhancement of the relaxation to β-adrenoceptor agonists in the PCA after pre-contraction with ET-1, and the palmar lateral vein after pre-contraction with U46619, cannot be due to inhibition of the pre-contraction response.

Another potential mechanism is through an interaction between ERK and the β-adrenoceptor signal transduction pathway. As the β-adrenoceptor is positively coupled to AC, its activation results in an elevation of intracellular cAMP and activation of PKA (Johnson, 2006). This signalling pathway plays a key role in relaxations induced by β-adrenoceptor agonists (Murray, 1990). In this study, relaxations induced by the AC activator forskolin were insensitive to PD98059, ruling out a direct role for ERK in the cAMP-dependent pathway to relaxation.

A further potential mechanism by which inhibition of ERK activation could enhance the relaxation to β-adrenoceptor agonists is through prevention of β-adrenoceptor desensitization. In a previous study on bovine tracheal smooth muscle, inhibition of protein kinase C activation with a non-selective protein kinase C inhibitor caused a similar enhancement of the relaxation to β-adrenoceptor agonists (Boterman *et al.*, 2006). The authors suggested that this was due to prevention of protein kinase C-induced receptor desensitization. However, we were unable to demonstrate any significant β-adrenoceptor desensitization in the PCA within the same time frame in which the relaxation to β-adrenoceptor agonists was carried out (data not shown), suggesting that prevention of receptor desensitization cannot explain the enhancement of the relaxation to β-adrenoceptor agonists by inhibition of ERK activation.

β-Adrenoceptors are also able to cause smooth muscle relaxation through AC-independent pathways. One such pathway is through activation of plasma membrane K^+ channels. In order to investigate whether inhibition of ERK activation enhances relaxations via the regulation of K^+ channel activation, we pre-contracted PCA segments with KCl. Raising extracellular K^+ in this way prevents efflux of K^+ through K^+ channels, and hence prevents any relaxation through this pathway. Under these conditions, inhibition of ERK activation with PD98059 had no effect on the relaxation to β-adrenoceptor agonists. An alternative explanation for why PD98059 does not enhance these relaxations after pre-contraction with KCl, but does after pre-contraction with U46619 or ET-1, could be that U46619 and ET-1 activate ERK, but KCl does not. However, the studies with the non-specific K^+ channel blocker TEA support the view that K^+ channels are involved. When K^+ channel activity was blocked with TEA, inhibition of ERK activation no longer resulted in an enhancement of relaxation responses. Therefore, taken together, these data suggest that ERK may be interacting with K^+ channels to impair relaxation to β-adrenoceptor agonists. Inhibition of ERK activation removes this impairment, thus enhancing these relaxations. Studies in other cell types have demonstrated that ERK can regulate K^+ channel activity (Li *et al.*, 2006), and ERK has been implicated in the impairment of K^+ channel activity during oxidative stress in blood vessels (Ross and Armstead, 2003). Therefore, it is not unreasonable to suggest this as a potential mechanism. Further studies are required to determine the exact nature of the K^+ channel involved in the relaxation to β-adrenoceptor agonists and how ERK modulates this channel.

β-Adrenoceptor-mediated vasodilatation is impaired in diseases such as hypertension and type 2 diabetes (Naslund *et al.*, 1990; Harada *et al.*, 1999; Chen and Doggrell, 2002; Grisk *et al.*, 2007). As ERK activity is enhanced in vascular smooth muscle in these diseases (Touyz *et al.*, 2002; Kim *et al.*, 2005; Matsumoto *et al.*, 2006), the data presented here open up the possibility that the impairment of β-adrenoceptor function is due in part to the increased ERK activity.

In summary, we have shown that inhibition of ERK activation enhances relaxation to β-adrenoceptor agonists in the PCA and the porcine palmar lateral vein. This enhancement does not appear to be due to a prevention of receptor desensitization or enhancement of a cAMP-dependent response. However, we provide evidence to suggest that inhibition of ERK activation enhances relaxation through enhancement of K^+ channel activity.

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Conflict of interest

None.

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