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Role of cytosolic phospholipase A_2 in the enhancement of α_2-adrenoceptor-mediated vasoconstriction by the thromboxane-mimetic U46619 in the porcine isolated ear artery: Comparison with vasopressin-enhanced responses

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Abstract

Pre-contraction with the thromboxane-mimetic U46619 enhances the subsequent α_2-adrenoceptor-mediated vasoconstriction in the porcine ear artery through an enhanced activation of ERK-MAP kinase. In this study we determined the role of $cPLA_2$ in this enhanced response, and determined whether vasopressin is also able to enhance α_2-adrenoceptor-mediated vasoconstriction through the same pathway. The $cPLA_2$ inhibitors AACOCF3 (50 μM) and MAFP (50 μM) both inhibited the U46619-enhanced α_2-adrenoceptor response, but had no effect on the direct α_2-adrenoceptor response. AACOCF3 also inhibited the enhanced ERK activation associated with the enhanced α_2-adrenoceptor-mediated vasoconstriction. Pre-contraction with arachidonic acid mimicked the effect of U46619 by enhancing the contractile response to the α_2-adrenoceptor agonist UK14304 (1 μM) and enhancing the α_2-adrenoceptor-mediated ERK activation. Pre-contraction with vasopressin also enhanced the contractile response to UK14304, but neither PD98059 (50 μM) nor AACOCF3 (50 μM) had any effect this vasopressin-enhanced response, indicating that neither the ERK pathway, nor $cPLA_2$ are involved in vasopressin-enhanced responses. The α_2-adrenceptor-stimulated activation of ERK was also unaffected by pre-contraction with vasopressin. On the other hand, inhibition of PKCζ inhibited the enhanced α_2-adrenoceptor contraction after pre-contraction with both U46619 and vasopressin. This study demonstrates that α_2-adrenoceptor-mediated vasoconstriction can be enhanced through two different pathways—one dependent upon the enhanced activation of ERK-MAP kinase through activation of $cPLA_2$, and the other through a different, ERK/$cPLA_2$-independent pathway.
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1. Introduction

α_2-Adrenoceptors cause vasoconstriction in porcine blood vessels through an ERK-MAP kinase pathway [1,2]. In the porcine ear artery, the α_2-adrenoceptor-mediated vasoconstriction is enhanced by pre-contraction with the thromboxane-mimetic U46619 [2] This enhancement occurs through an increased activation of ERK-MAP kinase, and is also dependent upon influx of extracellular calcium [2]. How ERK-MAP kinase activity is enhanced is not known.

Phospholipase A_2 (PLA_2) represents a family of structurally heterogeneous enzymes that catalyses hydrolysis of membrane phosphoplipids to liberate potent second messengers such as arachidonic acid, a precursor of eicosanoids including prostaglandins and leukotrienes [3]. Cytosolic PLA_2 ($cPLA_2$) is activated by calcium and is upstream of ERK [3,4]. It has been shown previously that arachidonic acid released by $cPLA_2$ is responsible for ERK activation in vascular smooth muscle cells. This can occur either through activation of protein kinase C (PKC), or

Abbreviations: AEBSF, 4-(2-aminoethyl)benzenesulphonyl fluoride; ANOVA, analysis of the variance; E-64, trans-epoxysuccinyl-L-leucylamide-(4-guanidino) butane; ERK, extracellular signal-regulated kinase; HELSS, Haloenol lactone suicide substrate; MAP kinase, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; PKC, protein kinase C; PLA_2, phospholipase A_2; TBS-T, tris-buffered saline containing 0.1% tween-20

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through the products of lipoxygenase metabolism of arachidonic acid [4,5]. Arachidonic acid metabolites have also been implicated in the enhancement of vasoconstriction in rat and hamster blood vessels [6,7]. Therefore, $cPLA_2$ may form an important link between the influx of calcium, and activation of ERK leading to the enhanced α_2-adrenoceptor-induced vasoconstriction.

In order to try to understand the mechanisms further it is necessary to determine if the enhancement is specific to U46619 or can be mimicked by other vasoconstrictors as well. Vasopressin is a potent vasoconstrictor, and has been shown to enhance adrenergic vasoconstriction in a number of blood vessels, although the adrenoceptor subtype was not identified [8–10]. This enhancement was shown to be dependent upon influx of extracellular calcium, in a manner similar to the U46619-enhanced α_2-adrenoceptor-mediated vasoconstriction. Therefore, it is possible that vasopressin is also able to enhance α_2-adrenoceptor-mediated vasoconstriction in the porcine ear artery through a similar pathway.

The aim of this study was to determine the role of $cPLA_2$ and arachidonic acid in the U46619-enhancement of the α_2-adrenoceptor-mediated vasoconstriction. The vasopressin-enhanced α_2-adrenoceptor response was used as a comparison. In the rabbit femoral artery and portal vein, arachidonic acid released by $cPLA_2$ is thought to cause calcium sensitisation through activation of the atypical protein kinase C isozyme PKCζ [11]. Therefore, a further aim was to determine whether inhibition of PKCζ could also inhibit the enhanced α_2-adrenoceptor-mediated vasoconstriction.

2. Methods

2.1. Isometric tension recordings

Porcine ears were obtained from a local abattoir and transported to the laboratory on ice. Ear arteries were dissected out and placed in Krebs–Henseleit buffer containing 2% Ficoll which had been pre-gassed with 95% O_2/5% CO_2, and stored overnight at 4 °C. The following day ear arteries were dissected into 5 mm ring segments and suspended in an isolated organ bath containing Krebs–Henseleit buffer maintained at 37 °C and constantly gassed with 95% O_2/5% CO_2. The lower support was fixed and the upper support was connected to a force transducer (Lectromed, Letchworth, UK) linked to a PCLab data acquisition system (AD Instruments Ltd., Hastings, UK) via an amplifier. After a 20 min equilibration period, tension was applied to the tissue, which was allowed to relax to a final resting tension of between 0.5 and 1 g wt. Before each experiment the tissues were contracted at least three times with 60 mM KCl, until the final two responses to KCl differed by less than 10%. Contractile responses were expressed as a percentage of the final KCl response.

2.2. Effect of pre-contraction on α_2-adrenoceptor-mediated vasoconstriction

Tissues were pre-contracted with the thromboxane-mimetic U46619 (1–5 nM), vasopressin (1–40 nM) or arachidonic acid (400 μM) as appropriate. The degree of pre-contraction obtained under these conditions was between 10 and 20% of the response to 60 mM KCl. Tissues were then exposed to a single, near maximal concentration of the α_2-adrenoceptor agonist UK14304 (1 μM) [12]. UK14304 causes contractile responses in the porcine ear artery through activation of α_2-adrenoceptors [13]. We have shown previously that pre-contraction enhances the UK14304 response over the whole range of the concentration–response curve [2]. However, as the contractile responses to the lower concentrations of UK14304 were difficult to measure accurately, particularly in the absence of pre-constrictor agent, we decided to use a near maximum concentration of UK14304 (1 μM). Contractions to UK14304 were measured from the pre-UK14304 level of tone. In control tissues UK14304 was added in the absence of pre-contraction. In some experiments inhibitors (50 μM PD98059; 10 μM PKCζ pseudosubstrate inhibitor; 50 μM MAFP; 10 μM HELSS; 10 μM NDGA; 10 μM ETI; 10 μM indomethacin) were added 1 h prior to addition of UK14304 or pre-contraction/UK14304. In the experiments carried out in the presence of 50 or 100 μM AACOCF3, the inhibitor was added 3 h prior to addition of UK14304 or pre-contraction/UK14304. Control tissues received the appropriate vehicle (0.1% DMSO for PD98059, HELSS, and NDGA, 0.18% for 50 μM AACOCF3, 0.36% for 100 μM AACOCF3, 0.1% ethanol for ETI, indomethacin and 0.3% ethanol for MAFP).

2.3. Concentration–response curves to UK14304 after pre-contraction with vasopressin

Owing to the transient nature of the vasopressin responses it was necessary to perform non-cummulative response curves to UK14304. Tissues were pre-contracted with vasopressin to between 10 and 20% of the KCl response, and then a single concentration of UK14304 added (10 nM to 10 μM). After each response, tissues were washed out and allowed to recover for 30 min prior to the next addition.

2.4. Effect of PD98059 or MAFP on U46619 and vasopressin responses

Concentration–response curves to U46619 were carried out in the absence or presence of PD98059 (50 μM) or MAFP (50 μM) in order to determine the role of ERK or $cPLA_2$, respectively. The effects of PD98059 or MAFP on the response to vasopressin were also determined. However, owing to the fact that the vasopressin-induced contractions were transient, especially at higher

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