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Shear stress sensitizes TRPV4 in endothelium-dependent vasodilatation

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ABSTRACT

The aim of this study was to better understand the role of TRPV4 in the regulation of blood vessel dilatation by blood flow and activation of GPCRs. Using pressure myography, the dilator responses to the TRPV4 agonist GSK1016790A and to acetylcholine, were examined in rat cremaster arterioles exposed to either no shear stress or to 200 µl/min flow for 6 min. In control vessels GSK1016709A caused vasodilatation (pEC_{50} 7.73 ± 0.12 M, ΔD_{max} 97 ± 3%) which was significantly attenuated by the TRPV4 antagonists GSK2193874 (100 nM) (pEC_{50} 6.19 ± 0.11 M, $p < 0.05$) and HC067047 (300 nM) (pEC_{50} 6.44 ± 0.12 M) and abolished by removal of the endothelium. Shear conditioned arterioles were significantly more sensitive to GSK1016790A (pEC_{50} 8.34 ± 0.11, $p < 0.05$). Acetylcholine-induced vasodilatation (pEC_{50} 7.02 ± 0.07 M, ΔD_{max} 93 ± 2%) was not affected by shear forces (pEC_{50} 7.08 ± 0.07 M, ΔD_{max} 95 ± 1%). The dilator response to acetylcholine was unaffected by the TRPV4 antagonist GSK2193874 in control arterioles (pEC_{50} 7.24 ± 0.07 M, ΔD_{max} 97 ± 2%). However, in shear treated arterioles, the acetylcholine-response was significantly attenuated by GSK2193874 (pEC_{50} 6.25 ± 0.12 M, $p < 0.05$) indicating an induced interaction between TRPV4 and muscarinic receptors. TRPV4 antibodies localized TRPV4 to the endothelium and shear stress had no effect on its localisation. Finally, agonist activation of the M3 muscarinic receptor opened TRPV4 in HEK293 cells.

We concluded that shear stress increases endothelial TRPV4 agonist sensitivity and links TRPV4 activation to muscarinic receptor mediated endothelium-dependent vasodilatation, providing strong evidence that blood flow modulates downstream signalling from at least one but not all GPCRs expressed in the endothelium.

1. Introduction

Blood vessels exist in a state of partial contraction, with the vessel tone produced by opposing dilator and constrictor factors, blood pressure and flow acting upon the vessel. Other factors, such as age, physical activity, obesity and blood glucose levels can also alter this balance and perturbed tone can lead to vascular dysfunction and disease [1]. The endothelial cell lining of blood vessels acts as a key regulator of vascular tone by producing different constrictor and/or dilator factors in response to a wide range of chemical and physical stimuli. The properties of the endothelial cells differ depending on their site in the vascular network. Endothelium-derived nitric oxide is the predominant dilator in larger arteries while in smaller arterioles, endothelium-dependent hyperpolarisation mediated *via* the calcium sensitive potassium channels (K_{Ca}), causes a greater contribution to smooth muscle relaxation [2].

The non-selective ion channel transient receptor potential vanilloid 4 (TRPV4) responds to a diverse range of stimuli encountered by the endothelium [3,4], such as hypo-osmolarity [5], shear stress [6,7] and pressure [8]. The endothelium is exposed to many of these stimuli. Endothelial dysfunction resulting from increased age [9], stenosis [10], hypertension [11] and Alzheimer's disease [12] is characterized by impaired endothelial responses to a number of TRPV4 activating stimuli and aberrant TRPV4 function is implicated in all of these conditions.

The effects of TRPV4 activation have been studied in a range of vascular systems. Agonist activation of TRPV4 with GSK1016790A or 4α-phorbol 12,13-didecanoate, leads to the vasodilatation of pre-constricted mesenteric arterioles [13]. TRPV4-dependent vasodilatation is linked to endothelium-dependent hyperpolarisation and leads to the opening of calcium-dependent potassium channels (K_{Ca}) that hyperpolarize the membrane of vascular smooth muscle cells and cause relaxation independently of the release of nitric oxide or cyclooxygenase

Abbreviations: ACh, acetylcholine; GPCR, G protein-coupled receptor; K_{Ca}, calcium–activated potassium ion channel; NOS, nitric oxide synthase; PAR_2-AP, protease-activated receptor 2 activating peptide (NH_2-SLIGRL-COOH); ROS, reactive oxygen species; TRPV4, transient receptor potential vanilloid 4

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products [13]. In human coronary arterioles, blood flow-induced vasodilatation is mediated by TRPV4, which is accompanied by reactive oxygen species generation [14]. The vasodilatation was inhibited by treatment with catalase, which hydrolyses hydrogen peroxide, suggesting that TRPV4 may be modulated by reactive oxygen species under some conditions. In pulmonary vascular beds, GSK1016790A caused a nitric oxide synthase (NOS)-dependent reduction of blood pressure, whereas in the systemic circulation, reduction of blood pressure due to GSK1016790A was not attenuated by NOS inhibition [15]. In addition to regulating vasodilation, TRPV4 activation can also trigger an endothelium-dependent contractile response in the aorta [16] and mesenteric arteries [7] while in pulmonary vessels TRPV4 mediated contraction is endothelium independent [17].

These observations show that TRPV4 activation has multiple effects regulating blood flow, with responses and mechanisms varying with the circulatory bed. It follows that understanding how signalling from blood flow and soluble factors can modulate TRPV4 opening, is key to understanding its function in the cardiovascular system.

There is mounting evidence for the sensitization and opening of TRPV4 by G protein-coupled receptor (GPCR) signalling and TRPV4 appears to be essential for some GPCR mediated signalling in blood vessels. An interaction between TRPV4 and the muscarinic receptor mediating endothelium-dependent relaxation to ACh is of particular interest. ACh-induced relaxation is impaired in mouse small mesenteric and cerebral vessels from TRPV4$^{-/-}$ mice or in the presence of a TRPV4 antagonist [12,18–20] however that is not the case in mouse carotid arteries [6,13,21,22] suggesting that artery size might influence the interaction between TRPV4 and the GPCR.

As noted, the complexity of the TRPV4 crosstalk with GPCRs to regulate vascular function in different circulatory beds is apparent because TRPV4 activation is tissue dependent and it is opened by distinct GPCRs in each tissue. [14,20,23–25]. For example in pulmonary smooth muscle cells TRPV4 was opened by serotonin receptors but not by α1 adrenoceptors or endothelin-1 receptors [24], while in endothelial cells TRPV4 was apparently opened by the muscarinic agonist acetylcholine but not by bradykinin [14]. In the aorta TRPV4 can be regulated by PAR_1, PAR_2 and angiotensin AT_1R signalling [16].

Shear stress from blood flow has been shown to dilate arterioles, and TRPV4 contributes to this effect [7]. Flow-dependent dilatation of carotid arteries is dependent on endothelial TRPV4 expression in mice whereas in the same experiments there was no role for TRPV4 in cholinergic dilatation [6]. Calcium influx through endothelial TRPV4 channels opens SK_{Ca} and IK_{Ca} channels to contribute significantly to endothelial mechanotransduction [6] with a small nitric oxide-dependent component [7]. Exposing human umbilical vein endothelial cells to shear stress increased TRPV4 expression at the cell surface and potentiated Ca^{2+} influx in response to the TRPV4 agonist GSK1016790A [26]. However, despite this evidence of functional interaction of GPCR signalling and shear stress on TRPV4, the physiological mechanisms of TRPV4 activation in endothelial cells remain poorly characterised. In particular, the effect of fluid flow on the sensitivity to GPCR-TRPV4 functional coupling is unknown.

We explored the function of TRPV4 in skeletal muscle resistance arterioles, by firstly characterising its function at steady state and secondly, by assessing how TRPV4 was integrated into endothelium-dependent vasodilatation after the endothelium was exposed to shear stress. We hypothesise that TRPV4 agonist sensitivity is altered by shear stress in the endothelium of resistance arterioles. Here we examine the effect of endothelial GPCR ligands and flow-induced shear stress on TRPV4-dependent dilatation of rat cremaster arterioles. The findings show how the endothelium could be communicating the effects of shear stress and endothelium-dependent vasodilatation by altering TRPV4 function.

2. Materials and methods

2.1. Chemicals and reagents

PAR_2–activating peptide SLIGRL-NH_2 (PAR_2-AP) was obtained from GL Biochem Ltd. (Shanghai, China). GSK1016790A, GSK2193874, HC067047, histamine, apamin, TRAM-34 and indomethacin were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia). Bovine serum albumin was from Bovogen (Keilor, Vic, Australia). All compounds were dissolved in water, except for GSK1016790A, GSK2193874 and HC067047 which were dissolved in dimethyl sulfoxide (DMSO) and L-NAME and indomethacin which were dissolved in 0.1 M sodium bicarbonate. Effectene transfection reagent was purchased from Qiagen (Frankfurt, Germany). The human M3 receptor with a C terminal DYKDDDDK tag cloned in pcDNA3.1 (M3R, coding sequence accession number XM_011544047.1) was purchased from Genscript (New Jersey, USA). Cell culture products: Dulbecco's modified eagle media (DMEM, Life Technologies, Mulgrave, Victoria, Australia), foetal bovine serum and hygromycin B from Sigma Aldrich (Castle Hill, NSW, Australia). Cell calcium imaging: Fura-2 and pluronic acid were purchased from Jomar Life Sciences (Adelaide, SA, Australia).

2.2. Pressure myography of cremaster arterioles

Adult male Wistar rats weighing between 200–400 g were killed using CO_2 asphyxiation, using protocols which complied with NHMRC requirements for the ethical treatment of animals and were approved by the RMIT University animal ethics committee. The cremaster muscle was removed and placed in modified Kreb's solution (in mM, KCl 5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, NaCl 111, $NaHCO_3$ 25.7, D-glucose 11.5 and HEPES 10) at 4 °C. The arterioles were dissected and cannulated between two glass pipettes with matching tip diameters of approximately 100 μm. Vessels were subjected to a pressure of 120 mmHg using a height adjustable reservoir, lengthened to remove lateral bowing and tested for leaks and were not used if they continued to display bowing or if they leaked. Once pressurised (70 mmHg), the vessels were warmed to 34 °C and allowed to spontaneously develop myogenic tone. Arterioles were considered suitable for further experimentation if they constricted by greater than 40% of the maximal diameter. Maximal diameter was determined by removing extracellular calcium, using Kreb's zero calcium solution ($CaCl_2$ 0 mM, EGTA 1 mM) at the end of each experiment. The lumen was filled with Kreb's solution containing 1% bovine serum albumin to protect the endothelium from damage during shear conditioning. Kreb's solution was superfused through the baths at approximately 4 ml/min, all inhibitors were placed in the superfusate and allowed to equilibrate for 10 min prior to administration of agonists. All agonists were added cumulatively to the bath every 2 min, using a 1:1000 dilution. In the event that two concentration effects curves were performed the entire bath volume was replaced twice and vessels were allowed to re-generate myogenic tone before the response curve was generated.

When needed, the endothelium was removed by passing an air bubble through the lumen 3–6 times until the vessels no longer responded to ACh (10 μM), after concentration effects response curves were determined, vessels were maximally dilated to Kreb's zero calcium solution. Shear stress was generated on the vessels by using a pressure regulated-servo controlled pump (Living Systems) at the distal end of the arterioles. To generate shear stress, a pressure difference of 60 mmHg across the vessels was used the input pressure was increased from 70 to 100 mmHg and the distal pressure was decreased to 40 mmHg thus maintaining intraluminal pressure in the vessel at approximately 70 mmHg. To cause shear, arterioles were exposed to an average flow rate of 200 μl/min for 6 min and flow was then stopped and the arterioles allowed to re-generate myogenic tone for 2–10 min prior to testing agonist responses as described above.