Vascular Pharmacology 74 (2015) 130–138

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Activation of endothelial IK_{Ca} channels underlies NO-dependent myoendothelial feedback

Paul M. Kerr [a], Ran Wei [f], Raymond Tam [f], Shaun L. Sandow [b], Timothy V. Murphy [c], Katarina Ondrusova [f], Stephanie E. Lunn [f], Cam Ha T. Tran [d], Donald G. Welsh [d], Frances Plane [e,f,*]

[a] Faculty of Health and Community Studies, MacEwan University, Robbins Health Learning Centre, Edmonton, Alberta T5J 4S2, Canada
[b] Inflammation and Healing Cluster, Faculty of Science, Health, Education and Engineering, University of the Sunshine Coast, Maroochydore, Queensland 4558, Australia
[c] Department of Physiology, School of Medical Sciences, University of New South Wales, Sydney, NSW2052, Australia
[d] Hotchkiss Brain and Libin Cardiovascular Research Institutes, Department of Physiology & Pharmacology, University of Calgary, AlbertaT2N-4N1, Canada
[e] Cardiovascular Research Centre, University of Alberta, Edmonton, AlbertaT6G 2H7, Canada
[f] Department of Pharmacology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AlbertaT6G 2H7, Canada

ARTICLE INFO

Article history:
Received 1 March 2015
Received in revised form 26 August 2015
Accepted 7 September 2015
Available online 9 September 2015

Keywords:
Myoendothelial feedback
NO
IK_{Ca} channel
IP_3 receptor
TRPC3

ABSTRACT

Agonist-induced vasoconstriction triggers a negative feedback response whereby movement of charged ions through gap junctions and/or release of endothelium-derived (NO) limit further reductions in diameter, a mechanism termed myoendothelial feedback. Recent studies indicate that electrical myoendothelial feedback can be accounted for by flux of inositol trisphosphate (IP_3) through myoendothelial gap junctions resulting in localized increases in endothelial Ca^{2+} to activate intermediate conductance calcium-activated potassium (IK_{Ca}) channels, the resultant hyperpolarization then conducting back to the smooth muscle to attenuate agonist-induced depolarization and tone. In the present study we tested the hypothesis that activation of IK_{Ca} channels underlies NO-mediated myoendothelial feedback. Functional experiments showed that block of IP_3 receptors, IK_{Ca} channels, gap junctions and transient receptor potential canonical type-3 (TRPC3) channels caused endothelium-dependent potentiation of agonist-induced increase in tone which was not additive with that caused by inhibition of NO synthase supporting a role for these proteins in NO-mediated myoendothelial feedback. Localized densities of IK_{Ca} and TRPC3 channels occurred at the internal elastic lamina/endothelial-smooth muscle interface in rat basilar arteries, potential communication sites between the two cell layers. Smooth muscle depolarization to contractile agonists was accompanied by IK_{Ca} channel-mediated endothelial hyperpolarization providing the first demonstration of IK_{Ca} channel-mediated hyperpolarization of the endothelium in response to contractile agonists. Inhibition of IK_{Ca} channels, gap junctions, TRPC3 channels or NO synthase potentiated smooth muscle depolarization to agonists in a non-additive manner. Together these data indicate that rather being distinct pathways for the modulation of smooth muscle tone, NO and endothelial IK_{Ca} channels are involved in an integrated mechanism for the regulation of agonist-induced vasoconstriction.

1. Introduction

Appropriate control of vascular tone and, thus of blood flow, pressure and tissue perfusion relies on coordinated behavior between smooth muscle and endothelial cells within the vessel wall mediated by gap junctions and release of paracrine agents [1–4]. Agonist-induced vasoconstriction is limited by movement of charged ions through gap junctions to hyperpolarize the smooth muscle cells and/or release of endothelium-derived nitric oxide (NO) [5,6]. This myoendothelial feedback mechanism is particularly important in resistance arteries where smooth muscle-endothelial cell coupling is crucial for blood flow regulation [7].

Early investigations into the basis of myoendothelial feedback suggested that agonist-induced increases in smooth muscle Ca^{2+} drive a global increase in endothelial Ca^{2+} to cause widespread activation of downstream targets [8–10]. In contrast, recent evidence suggests that discrete compartmentalization of temporally distinct Ca^{2+} signals and effectors rather than global Ca^{2+} changes may underpin the specificity and magnitude of endothelium-dependent control of vascular tone [2,11–13]. In this regard, structural specializations within the arterial wall of some arteries support a model of myoendothelial feedback in which flux of inositol trisphosphate (IP_3) through gap junctions activates localized endothelial Ca^{2+} signaling microdomains to activate

* Corresponding author at: Department of Pharmacology, 9-62 Medical Sciences Building, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.
E-mail addresses: kerrp7@macewan.ca (P.M. Kerr), rwei1@ualberta.ca (R. Wei), rtam@ualberta.ca (R. Tam), ssandow@usc.edu.au (S.L. Sandow), tim.murphy@unsw.edu.au (T.V. Murphy), ondrusov@ualberta.ca (K. Ondrusova), slunn@ualberta.ca (S.E. Lunn), chttran@ucalgary.ca (C.H.T. Tran), dwelsh@ucalgary.ca (D.G. Welsh), fplane@ualberta.ca (F. Plane).

http://dx.doi.org/10.1016/j.vph.2015.09.001
1537-1891/

Ca^{2+}-dependent effectors and initiate the negative feedback response [1,14–16]. Specifically, in rat mesenteric resistance arteries and hamster retractor feed arteries myoendothelial gap junction connexins and intermediate conductance calcium-activated potassium (IK_{Ca}) channels are in close spatial association with IP_3 receptors and endoplasmic reticulum within endothelial projections which extend through the internal elastic lamina to make contact with smooth muscle cells [2,11,17,18]. Functional evidence that this pathway mediates electrical myoendothelial feedback has come from experiments conducted in hamster retractor muscle feed arteries. The α_1-adrenoceptor agonist phenylephrine induced localized, IP_3-mediated Ca^{2+} signaling within endothelial projections and block of endothelial IK_{Ca} channels enhanced smooth muscle depolarization and vasoconstriction supporting their role of primary effectors in these vessels. However, changes in endothelial cell membrane potential were not assessed [17].

In addition to electrical myoendothelial feedback, in many arteries agonist-stimulated increases in tone are modulated by release of endothelium-derived NO [6,8,19] but the mechanisms underlying NO-dependent myoendothelial feedback have not been investigated. The present study was conducted to test the hypothesis that activation of IK_{Ca} channels also underpins NO-dependent myoendothelial feedback. Non-selective cation channels, transient receptor potential canonical type-3 (TRPC3) channels have been localized at myoendothelial junctions within rat mesenteric arteries and may provide a regenerative mechanism for refilling of Ca^{2+} stores and/or direct activation of IK_{Ca} channels [2,18]. Thus, we also addressed the role of TRPC3 channels in NO-mediated myoendothelial feedback.

The rat basilar artery was chosen for this study as acetylcholine-evoked changes in tone are blocked by inhibitors of NO synthase indicating that they can be fully accounted for by the release of NO [20]. For comparison, some experiments were also conducted in rat mesenteric arteries in which both NO and NO-independent smooth muscle hyperpolarization contribute to endothelium-dependent modulation of tone [21].

2. Materials and methods

Male Sprague–Dawley rats (250-350 g) were housed and euthanized by isofluorane inhalation followed by decapitation according to the standards of the Canadian Council on Animal Care and protocols approved by the Animal Care Committee of the Faculty of Medicine and Dentistry, University of Alberta. The brain and mesenteric bed were removed and placed into ice-cold Krebs buffer containing (mM): NaCl 119.0, $NaHCO_3$ 25.0, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.18, glucose 11, disodium EDTA 0.027 and $CaCl_2$ 2.5.

2.1. Wire myography

The basilar and third order mesenteric arteries were cleaned of adhering tissue, cut into segments (~2 mm in length) as previously described [20,22]. Briefly, arterial segments were mounted between two gold-plated tungsten wires (20 μm diameter) in a Mulvany–Halpern myograph (model 400A, J.P. Trading, Denmark). Changes in isometric tension were recorded via a PowerLab acquisition system using Chart 5.0 software (AD Instruments, Colorado, USA). Tissues were maintained in Krebs' buffer gassed with 95% O_2/5% CO_2 at 37 °C and were set to a pre-determined optimal resting tension of 2 mN for the basilar artery [20] and 5 mN for mesenteric arteries [22]. These values were determined from active length-tension curves using repeated stimulations with 5-HT and phenylephrine, respectively. In some experiments the endothelium was removed by gently rubbing the vessel lumen with a hair. Endothelial function was assessed in each tissue by application of either 5-HT (3 μM; basilar arteries) or phenylephrine (3 μM; mesenteric arteries) to induce a stable increase in tone (75% of maximal) followed by acetylcholine (10 μM). Tissues in which relaxation to acetylcholine was >90% of induced tone were deemed to have an intact endothelium and vessels in which the maximal relaxation to acetylcholine was <10% were deemed to be endothelium-denuded. Cumulative concentration-response curves to 5-HT (1 nM–10 μM) or phenylephrine (1 nM–10 μM) were constructed and after a washout period of 30 min, repeated in the presence of inhibitors. Results are expressed as % of the maximum response to agonists under control conditions.

2.2. Pressure myography

2–3 mm leak-free sections of basilar or third order mesenteric arteries were cleaned of adhering connective tissue and mounted in a pressure myograph as previously described [23]. Briefly, vessels were mounted between two glass micropipettes in an arteriograph chamber (Living Systems Instrumentation, Vermont, USA) filled with Krebs' solution and secured with thin monofilament sutures. The arteriograph was placed on the stage of an inverted microscope (Eclipse TE300, Nikon, Japan) and connected to a peristaltic pump regulated by a pressure servo controller (Living Systems Instrumentation, St. Albans, Vermont, USA) to allow manipulation of intraluminal pressure. Throughout the experiments vessels were superfused at a rate of 5 mls min^{-1} with oxygenated Krebs buffer at 37 °C. Images of the vessel were captured using a Sony XC-73CE monochrome camera module and measurement of arterial diameter was via an automated video dimension analyzer (Living Systems Instrumentation, St. Albans, Vermont, USA). Vessel diameters and pressure measurements were recorded via a PowerLab acquisition system using Chart 5.0 software (AD Instruments, Colorado, USA). Vessels were pressurized to 40 mm Hg and allowed to rest for 30 min before cumulative concentration-response curves to 5-HT (1 nM–10 μM; basilar arteries) or phenylephrine (1 nM–10 μM; mesenteric arteries) were constructed by addition of the agonist to the superfusate. At this pressure development of myogenic tone was minimal, but vessels produced a robust constriction to agonists. Agonist concentration-response curves were repeated in the presence of inhibitors which were either added to the superfusate or infused intraluminally via a microcannula. At the end of each experiment, arteries were exposed to a Ca^{2+}-free Krebs' buffer to achieve maximal diameter. Results are expressed as changes in diameter (μm).

2.3. Electrophysiology

Measurements of smooth muscle and endothelial cell membrane potential of endothelium-intact arteries were made using sharp glass microelectrodes back-filled with 3 M KCl and with resistances of 60–100 MΩ as previously described [20]. Briefly, arteries were cut open longitudinally and pinned to the bottom of a Sylgard chamber with the endothelial surface uppermost for recording of endothelial cell membrane potential or endothelial surface downwards for recording of smooth muscle membrane potential and visualized using a binocular microscope. The criteria for successful cell impalement included 1) a sharp negative deflection upon entry, 2) a stable recording for ≥1 min following entry, and 3) a sharp return to baseline upon electrode removal. Tissues were maintained at 37 °C and constantly perfused with warmed Krebs buffer at a rate of 5 ml min^{-1}. Membrane potential measurements were recorded via a PowerLab acquisition system using Chart 5.0 software (AD Instruments, Colorado, USA). Drugs were added to the perfusate as indicated and the membrane potential values quoted were recorded within 1–2 min of each drug addition.

2.4. Immunohistochemistry

TRPC3 and IK1 (IK_{Ca}) distribution was determined using conventional confocal immunohistochemistry as previously described [18]. In brief, animals were perfused via the left ventricle with a clearance solution of 0.1% bovine serum albumin (BSA), 10 U/ml of heparin and 0.1% NaNO3 (as a supramaximal dilator) in saline, and subsequently fixed with 2% paraformaldehyde in 0.1 mM of PBS. To optimize the area