European Journal of Pharmacology 750 (2015) 108–117

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Cardiovascular pharmacology

Sex differences in the role of transient receptor potential (TRP) channels in endothelium-dependent vasorelaxation in porcine isolated coronary arteries

Pui San Wong, Richard E. Roberts, Michael D. Randall *

Pharmacology Research Group, School of Life Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, United Kingdom

ARTICLE INFO

Article history:
Received 24 November 2014
Received in revised form
12 January 2015
Accepted 15 January 2015
Available online 22 January 2015

Keywords:
Bradykinin
Endothelium-dependent relaxation
Sex differences
Transient receptor potential vanilloid type 4 channel (TRPV4)
Transient receptor potential canonical type 3 channel (TRPC3)
Hydrogen peroxide (H_2O_2)

Chemical compounds studied in this article:
A23187 (PubChem CID: 11957499)
Bradykinin (PubChem CID: 6026)
Ethyl 1-[4-(trichloroprop-2-enamido) phenyl]-5-(trifluoromethyl)-1H-pyrazole-4-carboxylate (Pyr3) (PubChem CID: 56964346)
Hydrogen peroxide (PubChem CID: 784)
Indomethacin (PubChem CID: 3715)
L-NAME (PubChem CID: 39836)
1-[2-(4-Methoxyphenyl)-2-[3-(4-methoxyphenyl)propoxy]ethyl]imidazole (SKF96365) (PubChem CID: 104955)
2-di(phenyl)boranyloxyethanamine (2-APB) (PubChem CID: 1598)
2-[3-(4-pentylphenyl)prop-2-enoylamino] benzoic acid (ACA) (PubChem CID: 1974)
2,4-dichloro-N-(propan-2-yl)-N-{2-[(propan-2-yl)amino]ethyl}benzene-1-sulfonamide (RN1734) (PubChem CID: 3601086)

ABSTRACT

Endothelial and smooth muscle Transient Receptor Potential (TRP) channels contribute to regulation of vascular tone. We have previously reported sex differences in the endothelial function in porcine isolated coronary arteries (PCAs). The present study examined the role of TRP channels in endothelium-dependent and H_2O_2-induced vasorelaxations in male and female PCAs. Distal PCAs were mounted in a wire myograph and precontracted with U46619. Concentration–response curves to bradykinin, H_2O_2 and A23187 were constructed in the presence of TRP channel antagonists with or without L-NAME and indomethacin to inhibit NO synthase and cyclooxygenase respectively. 2-APB (TRPC & TRPM antagonist) inhibited the maximum relaxation (R_{max}) of the bradykinin-induced vasorelaxation and abolished the EDH-type response in PCAs from both sexes. SKF96365 (TRPC antagonist) inhibited the R_{max} of bradykinin-induced vasorelaxation in males, and inhibited R_{max} of the EDH-type response in both sexes. Pyr3 (TRPC3 antagonist) inhibited both the NO and EDH components of the bradykinin-induced vasorelaxation in males, but not females. RN1734 (TRPV4 antagonist) reduced the potency of the NO component of the bradykinin-induced vasorelaxation in females only, but inhibited the R_{max} of the EDH-type component in both sexes. 2-APB, SKF96365 and RN1734 all reduced the H_2O_2-induced vasorelaxation, whereas Pyr3 had no effect. No differences in expression level of TRPC3 and TRPV4 between sexes were detected using Western blot. Present study demonstrated a clear sex differences in the role TRP channels where TRPC3 play a role in the NO- and EDH-type response in males and TRPV4 play a role in the NO-mediated response in females.

Abbreviations: A23187, calcium ionophore; ACA, 2-[3-(4-pentylphenyl)prop-2-enoylamino]benzoic acid; EDH, endothelium-derived hyperpolarization; H_2O_2, hydrogen peroxide; L-NAME, N^G-nitro-L-arginine methyl ester; PCAs, porcine coronary arteries; Pyr3, ethyl 1-[4-(trichloroprop-2-enamido)phenyl]-5-(trifluoromethyl)-1H-pyrazole-4-carboxylate; RN1734, 2,4-dichloro-N-(propan-2-yl)-N-{2-[(propan-2-yl)amino]ethyl}benzene-1-sulfonamide; SKF96365, 1-[2-(4-Methoxyphenyl)-2-[3-(4-methoxyphenyl)propoxy]ethyl]imidazole; TRP, Transient receptor potential; TRPC, transient receptor potential canonical channel; TRPM, transient receptor potential melastatin channel; TRPV, transient receptor potential vanilloid channel; U46619, 9,11-dideoxy-9α,11α-epoxymethanoprostaglandin $F_{2\alpha}$; 2-APB, 2-di(phenyl)boranyloxyethanamine

* Corresponding author. Tel.: +44 115 82 30185; fax: +44 115 82 30142.
E-mail address: michael.randall@nottingham.ac.uk (M.D. Randall).

http://dx.doi.org/10.1016/j.ejphar.2015.01.022
0014-2999/

1. Introduction

The transient receptor potential (TRP) channels are non-selective cation-permeable channels detected in endothelial and vascular smooth muscle cells, playing a role in the regulation of vascular tone (Bubolz et al., 2012; Earley and Brayden, 2010; Huang et al., 2011). Three subfamilies of the TRP proteins, TRPM (TRP *m*elastatin), TRPC (TRP *c*anonical) and TRPV (TRP *v*anilloid) channels, have been reported as mediators of oxidative stress (Balzer et al., 1999; Bubolz et al., 2012; Kraft et al., 2004; Poteser et al., 2006). Specifically, TRPM2 channel has been demonstrated to be activated by H_2O_2 (Bari et al., 2009; Hecquet et al., 2008), whereas endothelial TRPC3 and TRPC4 channels are redox-sensitive cation channels (Balzer et al., 1999; Poteser et al., 2006). Increased levels of H_2O_2 have been detected in various pathological diseases including essential hypertension in human subjects (Lacy et al., 2000) and ischaemia and reperfusion of rat brain (Hyslop et al., 1995). Therefore, further understanding of the mechanism of action of H_2O_2 involving TRP channels in vascular function may benefit the development of new strategies in treatment and prevention of diseases related to H_2O_2 (Burgoyne et al., 2013).

Other vasoactive substances which regulate vascular tone include endothelium-derived relaxing factor (NO), prostacyclin and endothelium-derived hyperpolarization (EDH)-type responses (Edwards et al., 2010; Furchgott and Zawadzki, 1980; Taylor and Weston, 1988). We have previously demonstrated clear sex differences in the endothelial function in rat mesenteric arterial bed and porcine distal coronary arteries (PCAs), where NO plays a more prominent role in males while the EDH-type response is greater in females (McCulloch and Randall, 1998; Wong et al., 2014c). This could be a possible explanation for the higher cardiovascular risk observed in men and postmenopausal women compared to pre-menopausal women (Luksha et al., 2009). However, most of the current studies on endothelial function are still being conducted on arteries from either males only or from either sexes and conclusions from these results may be biased and inconsistent as previously discussed (Wong et al., 2014c).

Previous studies on TRP channels have demonstrated sex differences, where inhibition of TRPM2 channels and knockdown of TRPM2 expression in mice have significantly protected male neurons from cell death, but had no effect in females (Jia et al., 2011). In the mouse bladder, a higher gene expression level of TRPV1 has been reported in female compared to male mice (Kobayashi et al., 2009). To our knowledge, no-one has yet investigated if there are sex differences in the role of endothelial TRP channels in vascular control. We have previously demonstrated sex differences in the endothelium-dependent relaxations to bradykinin in PCAs where endogenous H_2O_2 plays a role in females but not males (Wong et al., 2014b, c). Therefore, using pharmacological antagonists, the present study examined whether TRP channels contribute to sex differences in bradykinin-induced vasorelaxation specifically the role of TRPC3 and TRPV4 channels and also the roles of TRP channels in H_2O_2-mediated vasorelaxation in PCAs. To further evaluate the effects of non-selective TRP antagonist on endothelium-dependent but receptor independent vasorelaxant, A23187, a calcium ionophore was used.

2. Materials and methods

2.1. Tissue preparation

Hearts from male and female pigs (large white hybrids pigs, 4–6 months old, weighing ~50 kg) were collected from a local abattoir and transported to the laboratory in ice-cold modified Krebs'-Henseleit solution (118 mM NaCl, 4.8 mM KCl, 1.1 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 11.6 mM D-glucose, 1.25 mM $CaCl_2$) previously gassed with 5% CO_2 and 95% O_2. The distal part of the coronary artery was dissected and placed in 2% w/v Ficoll in Krebs'–Henseleit solution for overnight storage at 4 °C. The following day, tissues were finely dissected, cleaned of adherent connective and fatty tissues. PCAs cut into rings of about 2 mm in length with approximate diameter of 0.86 ± 0.02 mm in female and 0.89 ± 0.02 mm in male pigs were then mounted in a multichannel wire myograph (Model 610M, DMT, Aarhus N, Denmark) filled with 5 ml Krebs'-Henseleit solution gassed with 5% CO_2 and 95% O_2 and maintained at 37 °C. Seasonal variations in pig responses were not factored into the present study design but each set of experiment has been carried out with an internal contemporaneous control.

2.2. Experimental protocol

2.2.1. Wire myography

As previously described (Wong et al., 2014c), vessels were set to a baseline tension of 24.5 mN and left to equilibrate for approximately 30 min. Tension was measured and recorded using a PowerLab recording system (ADInstruments, Oxfordshire, UK). After 30 min of equilibration, responses to 60 mM KCl were determined twice. The vascular tone was then raised to about 40–90% of the second KCl contraction tone by the addition of the thromboxane A_2 mimetic, U46619 (1 nM–400 nM). Once stable tone was achieved, concentration–response curves to an endothelium-dependent vasorelaxant, bradykinin (0.01 nM–1 μM), A23187 (1 nM–3 μM) or H_2O_2 (1 μM–1 mM) were constructed in the presence of various inhibitors. N^G-nitro-L-arginine methyl ester (L-NAME) (300 μM) was used as a NO synthase inhibitor and indomethacin (10 μM) was used to inhibit the synthesis of prostanoids. To examine the role of TRP channels in H_2O_2-induced vasorelaxation and on endothelium-dependent vasorelaxation, the following inhibitors were used; 2-diphenylboranyloxyethanamine (2-APB) (10 μM or 100 μM) (Hagenston et al., 2009; Li et al., 2005; Togashi et al., 2008) and 2-[3-(4-pentylphenyl)prop-2-enoylamino]benzoic acid (ACA) (20 μM or 100 μM) (Bari et al., 2009; Kraft et al., 2006; Togashi et al., 2008) as non-selective TRP channels blockers. 1-[2-(4-methoxyphenyl)-2-[3-(4-methoxyphenyl)propoxy]ethyl]imidazole (SKF96365) (10 μM) (Huang et al., 2011) was used to inhibits TRPC channels whereas ethyl-1-[4-(2,3,3-trichloroprop-2-enoylamino)phenyl]-5-(trifluoromethyl)pyrazole-4-carboxylate (Pyr3) (3 μM) (Huang et al., 2011) and 2,4-dichloro-N-propan-2-yl-N-[2-(propan-2-ylamino)ethyl]benzenesulfonamide (RN1734) (30 μM) (Bagher et al., 2012; Bubolz et al., 2012) were used as selective TRPC3 and TRPV4 antagonist respectively. All inhibitors were added into the bath 1 h before pre-contraction with U46619. In the majority of cases, a higher concentration of U46619 was required to induce tone in the presence of TRP antagonists and in some cases the level of tone achieved with U46619 was slightly less than vehicle controls. Table 1A and B summarises the concentration of U46619 used and the level of tone induced under these conditions.

2.2.2. Western blotting

The relative expression levels of TRPC3 and TRPV4 in PCAs from male and female pigs were compared using western blotting. PCAs were finely dissected and cut into segments of approximately 1.5 cm in length. PCAs were then gassed with 5% CO_2 and 95% O_2 at 37 °C for 1 h in Krebs'-Henseleit solution as previously described (Wong et al., 2014c). Vessels (designated F1-F5 for PCAs from females and M1-M5 for PCAs from males) were homogenised on ice in lysis buffer (80 mM sodium β-glycerophosphate, 20 mM imidazole, 1 mM dithiothreitol, 1 mM sodium fluoride, pH7.6)