European Journal of Pharmacology 683 (2012) 197–203

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

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

Cardiovascular Pharmacology

Involvement of the peroxisome proliferator-activated receptor (PPAR) alpha in vascular response of endocannabinoids in the bovine ophthalmic artery

Maria Rosaria Romano, Marcello Diego Lograno *

Department of Pharmacobiology, University of Bari "Aldo Moro", Via Orabona 4, 70125 Bari, Italy

ARTICLE INFO

Article history:
Received 2 February 2012
Received in revised form 14 February 2012
Accepted 26 February 2012
Available online 10 March 2012

Keywords:
Anandamide
Palmitoylethanolamide
Ophthalmic artery
Peroxisome proliferators-activated receptor alpha
Nitric oxide

ABSTRACT

Endocannabinoids regulate vascular tone in a variety of vascular tissues. This study aimed to investigate the role of peroxisome proliferators-activated receptors (PPARs) in anandamide- and palmitoylethanolamide-induced relaxant responses on the bovine ophthalmic artery and to evaluate the mechanisms involved. The effects of anandamide and palmitoylethanolamide were examined under myographic conditions on arterial rings pharmacologically pre-contracted with 5-HT. Anandamide and palmitoylethanolamide relaxed the ophthalmic artery rings in time- and concentration-dependent manner stimulating the PPAR alpha (PPARα). The vasorelaxation to endocannabinoids was inhibited by PPARα antagonist GW6471 (1 μM), but not the PPAR gamma (PPARγ) antagonist GW9662 (1 μM). Anandamide-induced relaxation was attenuate during the first 60 min by AM251, a selective antagonist of cannabinoid CB_1 receptors, and Pertussis toxin, an inhibitor of $G_{i/o}$ protein; by the contrast, the palmitoylethanolamide-induced vasorelaxation was unaffected by cannabinoid antagonists and Pertussis toxin. Endothelium removal decreases slightly the potency and efficacy to endocannabinoids. The relaxant effect to anandamide and palmitoylethanolamide was inhibited by L-NMMA (300 μM), an inhibitor of nitric oxide synthase, and iberiotoxin (200 nM), a selective blocker of large conductance Ca^{2+}-activated K^+ (BK_{Ca}). These data support the view that anandamide and palmitoylethanolamide relax the ophthalmic artery in a time-dependent manner via the transcription factors PPARα suggesting a function for them in the physiological mechanisms of vascular regulation.

1. Introduction

Endocannabinoids are described as endogenous agonists of cannabinoid (CB) receptors (Pacher et al., 2006). They are considered unconventional neurotransmitters and are generated on demand in response to a rise in intracellular calcium or metabotropic receptor activation, rather than being stored in vesicles (Cadas et al., 1997). Anandamide was the first endocannabinoid identified (Devane et al., 1992), following numerous other N-acylethanolamines have been found in mammals (Lambert and Fowler, 2005). Emerging evidences suggest that endocannabinoid system is implicated in several physio-pathological processes; their effects are mostly explained via activation of the classical G-protein-coupled CB receptors and related downstream signaling cascades. However, studies carried out in knock-out mice have suggested the existence of new target sites for endocannabinoids such as transient receptor potential vanilloid type 1 (TRPV1) and nuclear receptor peroxisome proliferator-activated receptors (PPARs) (Pistis and Melis, 2010).

PPARs are members of the nuclear receptor superfamily, modulate the expression of numerous gene families and are regarded ligand-activated transcription factors (Touyz and Schiffrin, 2006). The PPAR family comprises three members α, γ, and β/δ, which are mainly involved in the energy homeostasis, in the regulation of metabolism and the modulation of cardiovascular system (Ferrè, 2004). At vascular level, PPARs influence oxidative stress, inflammatory process and cell growth, and appear to be implicated in the vasculoprotective effects. In particular, PPARα and PPARγ are widely expressed in the vascular smooth muscle and endothelial cell where they control the production of nitric oxide and the expression of endothelin-1 (Goya et al., 2004; Irukayama-Tomobe et al., 2004; Touyz and Schiffrin, 2006). Recently, it has also been shown that main constituent psychoactive of cannabis Δ^9-tetrahydrocannabinol can induce vasorelaxant responses through activation of PPARγ in different vascular bed (O'Sullivan et al., 2005, 2006). In addition, 2-arachidonylglycerol, noladin ether and virodhamine determine an increase of transcriptional PPARα activity (Kozak et al., 2002). N-arachidonyl dopamine and synthetic cannabinoids, WIN55212-2 and CP55940, are able to bind to PPARγ (O'Sullivan et al., 2009a). It has been demonstrated that anandamide directly activates some members of PPAR family like PPARα and PPARγ (Bouaboula et al., 2005; Sun et al., 2006). Palmitoylethanolamide that is a structurally similar compound to anandamide activates PPARα transcriptional activity (Lo Verme et al., 2005) and, more importantly, is devoid of cannabinoid-like activity (O'Sullivan, 2007). Numerous evidences

* Corresponding author. Tel./fax: +39 0805442797.
E-mail address: mdlograno@farmbiol.uniba.it (M.D. Lograno).

0014-2999/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2012.02.049

report that anandamide is a potent modulator of vascular tone and its mechanism of action is multi-factorial and depends on the species and preparation used (O'Sullivan et al., 2005; Romano and Lograno, 2006). In addition, it has been shown that anandamide induces PPARγ-mediated vasorelaxation of rat aorta but not palmitoylethanolamide (O'Sullivan et al., 2009a). Palmitoylethanolamide is categorized as an endocannabinoid-like molecule, able to exert direct vasorelaxation less potent than anandamide (Ho et al., 2008).

Therefore, the aim of the present study was to investigate whether anandamide and palmitoylethanolamide can induce a time- and concentration-dependent vasorelaxation through the activation of PPARs in the bovine ophthalmic artery and to examine the involved mechanisms.

2. Materials and methods

2.1. Tissue preparation

Experiments were in compliance with the European Community guidelines for the use of experimental animals and were approved by the institutional ethics committee. The technique for isolation and preparation of ophthalmic arterial rings has been performed as described previously by Romano and Lograno (2006). In brief, bovine eye, including the immediate retro-orbital structures, were obtained from a local abattoir within 5 min of slaughter and immediately put in ice-cold oxygenated modified Krebs physiological salt solution of the following composition (mM): NaCl (136.8), KCl (5.4), $MgSO_4$ (0.8), $NaHCO_3$ (12), $CaCl_2$ (2.7), D-glucose (5.0), Na-ascorbate (0.2), pH 7.4. The main ophthalmic artery running along the optic nerve to the eye was dissected and freed of surrounding connective and adipose tissue. Care was taken not to damage the luminal surface of the preparation. Two adjacent rings were cut from each artery (0.6–1.0 mm in diameter, 2–3 mm in length) and were mounted on fine tungsten wires on a miograph system (Fort 10, WPI, Sarasota, FL, USA) containing modified Krebs physiological salt solution. Tissues were maintained at 37 °C under a tension of 5 mN and gassed with a mixture of 95% O_2/5% CO_2. Changes in isometric tension were recorded using Chart 5 software.

2.2. Experimental protocols

The tissue was allowed to equilibrate for at least 90 min before each experiment. Rings were initially contracted with 5-hydroxytryptamine (5-HT) (1 μM) to increase tension. When a stable contraction was maintained, the vasorelaxant effect of a single concentration of endocannabinoid (palmitoylethanolamide or anandamide) or vehicle control on induced tone was assessed as the reduction in tone over time. For each experimental protocol, endocannabinoid-treated and vehicle control experiments were performed in adjacent segments of the same artery. In some experiments, the vascular effects of anandamide and palmitoylethanolamide were investigated in un-contracted vessels. In all vessels, the integrity of the endothelium was assessed by precontracting the vessels with 1 μM 5-HT followed by relaxation with 10 μM carbachol; relaxation greater than 60% were designated as endothelium-intact. In some arterial rings, endothelium was removed by gently rubbing the intimal surface of the vessel with a human hair; carbachol-induced relaxation of 10% indicated successful removal (Romano and Lograno, 2006).

To investigate any possible contribution of cannabinoid receptor activation, some experiments were performed in the presence of the cannabinoid CB_1 receptor antagonist AM251 (1 μM) or cannabinoid CB_2 receptor antagonist AM630 (1 μM), both added 10 min prior pre-contraction. To assess if anandamide and palmitoylethanolamide acts at a $G_{i/o}$-protein-coupled receptor, some arterial rings were pre-treated for 45 min with 500 ng/ml Pertussis toxin (Romano and Lograno, 2006), and the vasorelaxation to anandamide and palmitoylethanolamide or vehicle was investigated. The involvement of transient receptor potential vanilloid 1 (TRPV1) on sensory nerve was investigate by pre-treatment for 30 min with the TRPV1 agonist capsaicin (10 μM) to deplete the sensory nerve of vasoactive neurotransmitters (Zygmunt et al., 1999).

To evaluate the functional presence of PPARs in the bovine ophthalmic artery, some experiments were performed with the selective agonist of PPARα WY14643 (1 μM) and the selective agonist PPARγ ciglitazone (1 μM). The contribution of PPAR activation on the endocannabinoid-evoked vasorelaxant effects was investigated in the presence of the selective PPARα antagonist GW6471 (1 μM) or the selective PPARγ antagonist GW9662 (1 μM) both added 10 min prior pre-contraction.

In some preparations, the role of endothelium-derived nitric oxide was investigated by utilizing the nitric oxide synthase inhibitor N^G-monomethyl-*L*-arginine (*L*-NMMA, 300 μM; Romano and Lograno, 2006). An incubation of 20 min was used for the fatty acid amide hydrolase (FAAH) inhibitor URB597 (1 μM) before determination of anandamide and palmitoylethanolamide responses. Finally, the potassium channel role in the vasorelaxant responses to endocannabinoids was assayed by utilizing iberiotoxin (200 nM), glibenclamide (5 μM), apamin (100 nM) and 4-aminopyridine (1 mM).

2.3. Data analysis

All data were expressed as a mean percentage relaxation of 5-HT-induced tone, with error bars representing the mean ± S.E.M. R_{max} refers to the maximal relaxation achieved. Statistical analysis was performed using Student's *t*-test and when appropriate analysis of variance (ANOVA), followed by Bonferroni's post hoc test (GraphPad Prism 5.0). In all cases, n = the number of arteries from different animals. All differences were considered as statistically significant when $P<0.05$.

2.4. Materials

All drugs were purchased from Tocris Bioscience (Bristol, UK) except where indicated. Carbachol chloride, 5-HT creatinine sulfate, iberiotoxin and Pertussis toxin were obtained from Sigma Aldrich (St Louis, MO, USA). URB597 (3′-carbamoyl-biphenyl-3-yl-cyclohexylcarbamate) was obtained from Cayman Chemical (Ann Arbor, MI, USA). 4-aminopyridine, apamin, L-NMMA acetate, carbachol, and Pertussis toxin were dissolved in distilled water. Anandamide was supplied as a water-soluble emulsion and dissolved in distilled water. Capsaicin and glibenclamide were dissolved in ethanol to a stock concentration of 10 mM with further dilutions made daily in Krebs solution. AM251 (*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide), AM630 (6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl](4-methoxyphenyl)-methanone), GW6471 ([(2S)-2-[[(1Z)-1-methyl-3-oxo-3-[4-(trifluoromethyl) phenyl]-1-propenyl]amino]-3-[4-[2-(5-methyl-2-phenyl-4-oxazolyl)ethoxy]phenyl]propyl]-carbamic acid ethyl ester), GW9662 (2-chloro-5-nitro-*N*-phenylbenzamide), ciglitazone and WY14643 ([[4-chloro-6-[(2,3-dimethylphenyl)amino]-2-pyrimidinyl] thio]acetic acid) were dissolved in dimethylsulphoxide (Sigma) to 10 mM with further dilutions prepared daily in Krebs solution.

3. Results

3.1. Time-dependent vascular response to the anandamide in the bovine ophthalmic artery

The anandamide-induced relaxant effect at the concentration 100 nM became significantly different to the vehicle control from 90 min (2 h, R_{max} anandamide = 47.8 ± 6.3%, $n = 8$, *$P<0.05$,