European Journal of Pharmacology ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Cardiovascular pharmacology

Vasorelaxant effect of propentofylline in isolated equine digital veins

Nasr Kabbesh [a], Marc Gogny [a], Gérard Chatagnon [b], Jacques Noireaud [c], Jean-Claude Desfontis [a], Mohamed Yassine Mallem [a,*]

[a] LUNAM Université Oniris, "UPSP 5304 de physiopathologie animale et de pharmacologie fonctionnelle", Atlanpole-La Chantrerie, BP 40706, Nantes F-44307, France
[b] LUNAM Université Oniris, "UPSP SSBR, Sécurité Sanitaire en Biotechnologies de la Reproduction", Atlanpole-La Chantrerie, BP 40706, Nantes F-44307, France
[c] LUNAM Université Angers, Inserm UMR 1063, IBS-IRIS, rue des Capucins, F-49100 Angers, France

ARTICLE INFO

Article history:
Received 26 February 2013
Received in revised form
26 August 2013
Accepted 4 September 2013

Keywords:
Propentofylline
Endothelium
Vasorelaxation
EDVs
Nitric oxide

ABSTRACT

We evaluated the vasorelaxant effect of propentofylline (PPF), a methylxanthine derivative, and its mechanism of action in equine digital veins (EDVs). Cumulative concentration-response curves to PPF (1 nM–300 μM) were recorded in phenylephrine-precontracted EDV rings under different experimental conditions. PPF-induced relaxation was partially inhibited by endothelium removal, but was unaltered by CGS-15943 (an adenosine receptor antagonist; 3 μM). PPF-induced relaxation was partially inhibited in the presence of L-NAME (a nitric oxide (NO) synthase inhibitor; 100 μM), ODQ (an inhibitor of soluble guanylyl cyclase; 30 μM) or Rp-8-Br-PET-cGMP-S (a protein kinase G inhibitor; 3 μM). It was not modified by indomethacin (a non-selective cyclooxygenase (COX) inhibitor; 10 μM), and was slightly potentiated by H-89 (a protein kinase A inhibitor; 2 μM). In endothelium-intact EDVs, PPF-induced relaxation was associated with a 2.4- and 24.1-fold increase in the tissue cGMP and cAMP content respectively. PPF (100 μM) did not shift the concentration–response curve to phenylephrine (1 nM–300 μM) but reduced the maximal effect. To investigate whether PPF can affect cAMP- and cGMP-induced relaxations, relaxation curves to forskolin (an activator of adenylate cyclase) and to sodium nitroprusside (SNP, a NO donor) were recorded in EDV rings pretreated with PPF (100 μM). PPF only slightly potentiated the forskolin-induced relaxation without affecting the SNP-induced relaxation. We demonstrated that PPF-induced relaxation in EDVs is partially endothelium-dependent. The PPF-induced relaxation partially occurred via NO release and both cAMP and cGMP generation, through COX-independent mechanisms but could also result from the inhibition of cAMP-phosphodiesterase activity for the highest concentrations.

1. Introduction

Equine laminitis is considered as a complex disease involving various etiopathogenic factors (Bailey et al., 2004; Eades, 2010; Heymering, 2010, Katz and Bailey, 2012). Although the precise mechanisms involved in the pathogenesis of laminitis remain incompletely defined, several studies have indicated that equine laminitis may be related to the development of low blood flow and hypoxia in the digital circulation (Moore et al., 1989; Hood et al., 1993; Robertson et al., 2009). Acepromazine, which has vasodilatory property, has thus been used during the past two decades in the treatment of acute laminitis (Hunt et al., 1994; Slater et al., 1995, Ingle-Fehr and Baxter, 1999; Belknap, 2010), whereas other drugs, such as isoxsuprine have been shown to be ineffective vasodilators in the equine digit (Belknap, 2010). The digital vascular benefit of such drugs may be more questionable if we consider that their vasodilator effect results from pharmacological targeting of a single receptor. The latter is unlikely to be sufficient to increase the digital blood flow, and would be ineffective against pathological digital vasoconstriction which is caused by the activation of several different receptors (i.e. serotonin and endothelin receptors) (Katwa et al., 1999; Peroni et al., 2005; Peroni et al., 2006). Therefore, the possibility of a vasodilator that operates via a common secondary messenger system, rather than targeting a single membrane receptor, as this has been recently suggested (Mitchell and Elliott, 2012), is theoretically attractive. Methylxanthine derivatives, such as pentoxifylline (PTX) or propentofylline (PPF), which are non-specific inhibitors of phosphodiesterases (Schubert et al., 1997; Kruuse et al., 2001) could be a very interesting option. PTX and PPT have been reported to exert a wide range of pharmacological and clinically relevant properties including hemorheological (Seiffge and Katsuyoshi, 1985; Weiss et al., 1994; Belknap, 2010), anti-inflammatory (Milam et al., 1992; Barton et al.,

* Corresponding author. Tel.: +33 240 68 77 85; fax: +33 240 68 77 42.
E-mail address: yassine.mallem@oniris-nantes.fr (M.Y. Mallem).

0014-2999/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2013.09.003

1997; Sweitzer and De Leo, 2011) and vasodilatory effects (Kamphuis et al., 1994; Kruuse et al., 2001; Plaschke et al., 2001; Kabbesh et al., 2012). However, few studies have examined their benefit in the treatment of equine laminitis. PTX was reported to be unable to increase equine digital blood flow (Ingle-Fehr, and Baxter, 1999, Belknap 2010), although our previous work showed that PTX possesses vasorelaxant properties in isolated equine digital veins (EDVs) (Kabbesh et al., 2012).

Unlike PTX, there have been no studies into the effect of PPF on the equine digital blood flow. Although PPF has been shown to produce vasorelaxation in non-equine species (Hudlicka et al., 1981; Plaschke et al., 2001; Turcáni and Turèáni, 2001), only one study has reported that PPF produced a vasorelaxant effect in isolated equine digital vessels (Evans and Elliott, 1994), but the exact mechanism has yet to be elucidated. Although PPF is structurally similar to PTX, biochemical studies have reported differences in the PTX- and PPF-mediated inhibition of phosphodiesterase (PDE) activity (Meskini et al., 1994). Thus, one could postulate that different vasorelaxant effects may also occur in EDVs. The aim of our study was to investigate the mechanisms of action of PPF in healthy EDVs.

2. Materials and methods

2.1. Animals

At an abattoir (Cholet, France), distal portions of forelimbs were collected from 30 healthy adult horses immediately after euthanasia via stunning and exsanguination. Digital veins were quickly removed as close to the coronary band as possible and flushed with ice-cold Krebs solution containing 118.3 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 20 mM $NaHCO_3$, 0.016 mM EDTA, 11.1 mM glucose and 2.5 mM $CaCl_2$ and aerated with 95% oxygen and 5% carbon dioxide (pH 7.4). After collection, vessels were maintained in ice-cold Krebs solution for transport to the laboratory (30–35 min).

2.2. Tissue preparation and tension recording

In the laboratory, EDVs were cleared of loose connective tissue and cut into 3–4 mm rings. Some EDV rings were denuded of their endothelium by gently rubbing the intimal surface with a pair of small fine forceps. Thereafter, EDV rings were suspended between stainless steel wires in 5 mL organ baths containing Krebs solution, which were aerated with a gas mixture of 95% oxygen and 5% carbon dioxide and maintained at 37 °C (Mallem et al., 2003). Briefly, one side of each ring was attached to a force isometric transducer and the other side of the ring was fixed to the base of the organ bath and a basal resting tension of 2 g was then applied. Isometric tension changes were continuously measured by a force–displacement transducer (EMKA Technologies, Paris, France) and recorded on data acquisition software (Acqknowledge BIOPAC system, MP150, Cerom, Paris). After a 1 h equilibration period with the Krebs solution changed every 15 min, EDV rings were contracted with 2 μM phenylephrine, an α_1-adrenoceptor agonist. Once the contraction had reached a stable plateau, the function of endothelium was checked for the presence of at least 70% relaxation in response to acetylcholine (1 μM) for endothelium-intact EDV rings. The absence of the functional endothelium was confirmed, for endothelium-denuded EDV rings, by the inability of acetylcholine (1 μM) to induce a relaxation. Preparations failing to produce contraction to phenylephrine greater than 4.5 g were discarded.

2.3. Experimental design

After a second equilibration period of 30 min, EDV rings were again contracted with phenylephrine, and once the contraction had reached a plateau, cumulative concentration–response curves were performed under different experimental conditions with PPF (1 nM–300 μM), forskolin (a direct activator of adenylate cyclase, 1 nM–300 μM) or sodium nitroprusside (SNP, a nitric oxide (NO) donor, 1 nM–300 μM). Cumulative concentration–response curves to phenylephrine (1 nM–300 μM) were also constructed. Only one cumulative concentration–response curve to a vasoactive agent was obtained from any individual vessel ring. Within the same type of experiment, vessel segments isolated from the same horse were used as control and treated preparations.

2.3.1. Role of the endothelium and adenosine receptors in the PPF-induced relaxation

In a first set of experiments, we evaluated the endothelium/smooth muscle role in the PPF-induced relaxation. Cumulative concentration–response curves to PPF were constructed in EDV rings with or without endothelium. Since adenosine has been shown to be implicated in the effect of PPF (Parkinson et al., 1994), the participation of adenosine receptors in the PPF-induced relaxation was assessed on endothelium-intact EDVs using the adenosine receptor inhibitor, 9-chloro-2-(2-furanyl)-[1,2,4]triazolo [1,5-c]quinazolin-5-amine (CGS-15943) (3 μM) applied 30 min before constructing cumulative concentration–response curves to PPF.

2.3.2. Identification of cellular mediators in the PPF-induced relaxation

In a second set of experiments carried out to study the involvement of mediator factors, the effects of various inhibitors on PPF-induced vascular relaxation were examined. To determine the roles of NO and vasorelaxant cyclooxygenase (COX)-derived products (e.g. prostacyclin) in PPF relaxation, endothelium-intact EDV rings were treated for 30 min in the presence of Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME, a non-selective NO synthase (NOS) inhibitor, 100 μM), 1[H]-1,2,4-oxadiazolol[4,3,-a] quinoxalin-1-one (ODQ, an inhibitor of soluble guanylyl cyclase, 30 μM) or indomethacin (a non-selective COX inhibitor, 10 μM). Control EDV rings were not treated during that period. The role of protein kinase G (PKG) or protein kinase A (PKA) in the PPF-induced relaxation was also evaluated in endothelium-intact EDV rings incubated with of 2-bromo-3,4-dihydro-3-[3,5-O-[(R)-mercaptophosphinylide ne]-ß-D-ribofuranosyl]-6-phenyl-9H-imidazo [1,2-a]purin-9 (Rp-8-Br-PET-cGMP-S, a PKG inhibitor, 3 μM) or N-[2-[[3-(4-bromophenyl)-2-propenyl]amino]ethyl]-5-isoquinoline sulphonamide dihydrochloride (H-89, a PKA inhibitor, 2 μM) for 30 min. For each experimental condition, phenylephrine concentration was adjusted (0.3–2 μM) to induce a similar level of tone (5.5 ± 0.6 g) in EDV rings.

2.3.3. Effect of PPF on phenylephrine-induced contraction in endothelium-intact EDVs

In a third set of experiments, in order to determine whether PPF can interfere with phenylephrine-evoked contraction, the cumulative concentration–response curve to phenylephrine was performed on endothelium-intact EDV rings in the absence or in the presence of PPF (100 μM) applied 30 min before applying phenylephrine.

2.3.4. Effects of PPF on forskolin- and SNP-induced relaxations in endothelium-denuded EDVs

Finally, in order to test the hypothesis that PPF-induced inhibition of PDE activity might partially explain the vasorelaxant action

Please cite this article as: Kabbesh, N., et al., Vasorelaxant effect of propentofylline in isolated equine digital veins. Eur J Pharmacol (2013), http://dx.doi.org/10.1016/j.ejphar.2013.09.003