European Journal of Pharmacology 794 (2017) 209–215

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Full length article

The endogenous lipid N-arachidonoyl glycine is hypotensive and nitric oxide-cGMP-dependent vasorelaxant

Yousuf M. Al Suleimani*, Ahmed S. Al Mahruqi

Department of Pharmacology and Clinical Pharmacy, College of Medicine and Health Sciences, Sultan Qaboos University, 35, Alkoudh 123, Sultanate of Oman

ARTICLE INFO

Keywords:
N-arachidonoyl glycine
Vasorelaxation
Hypotensive
Nitric oxide
Na^+/Ca^{2+} exchanger

ABSTRACT

N-arachidonoyl glycine (NAGLY), is the endogenous lipid that activates the G protein-couple receptor 18 (GPR18) with vasodilatory activity in resistance arteries. This study investigates its hemodynamic effects and mechanisms of vasorelaxation. Hemodynamic effects of NAGLY in rats were assessed using a Biopac system and its vascular responses were assessed using a wire myograph. NAGLY (1 mg/kg) decreased blood pressure by 69.4 ± 5.5% and reduced renal blood flow by 88 ± 12% and the effects were not sensitive to inhibition by O-1918 (3 mg/kg). In resistant vessels, NAGLY (1–30 μM) induced concentration- and endothelium-dependent vasorelaxation and the effect was inhibited by the nitric oxide synthase inhibitor, L-NAME (300 μM), a cGMP synthase inhibitor, ODQ (10 μM), the antagonists of "endothelial anandamide" receptor, rimonabant (3 μM) and O-1918 (10 μM) and the inhibitor of Na^+/Ca^{2+} exchanger (NCX), KB-R7943 (10 μM). On the other hand, NAGLY-induced vasorelaxation was not affected by CID 16020046 (GPR55 antagonist), AM 251 (cannabinoid CB_1 receptor antagonist), AM 630 (cannabinoid CB_2 receptor antagonist), capsazepine (TRPV1 antagonist), indomethacin (cyclooxygenase inhibitor), TRAM34 (IKCa channel blocker), iberiotoxin (BKCa channel blocker) and GW9662 (PPARγ antagonist). At low concentrations of carbachol, NAGLY potentiated carbachol-induced vasorelaxation. NAGLY is an endothelium-dependent vasodilator and hypotensive lipid. The vasorelaxation is predominantly via activation of nitric oxide-cGMP pathway and NCX and probably mediated by the "endothelial anandamide" receptor, while the hypotensive effect of NAGLY appears not to involve the anandamide receptor. NAGLY also potentiates carbachol-induced vasorelaxation, the mechanism of which might involve stimulation of NO release.

1. Introduction

Since the identification of arachidonoyl ethanolamide (anandamide) as an endogenous agonist of the endocannabinoid receptor CB_1(Devane et al., 1992), a large number of endogenous long-chain *N*-acyl amino acids have been identified in the mammalian body including, for instance, *N*-arachidonoyl serine, *N*-arachidonoyl glycine (NAGLY), *N*-acyl taurines and oleoyl serine (see Hanus et al. (2014) for review). Recent studies have shown that some of these endogenous lipids with structure similar to anandamide might have vasoactive actions. Of particular interest, NAGLY which has attracted much attention as GPR18 receptor-mediated anti-nociceptive and anti-inflammatory agent (Bradshaw et al., 2009; Jeong et al., 2010), has also reported to have vascular activity *in vitro* (Bondarenko et al., 2013; Parmar and Ho, 2010).

NAGLY is produced from anandamide *via* two distinct pathways including oxidative metabolism of the ethanolamine moiety of anandamide and conjugation of glycine to arachidonic acid, which is released during anandamide hydrolysis by fatty acid amide hydrolase (Bradshaw et al., 2009). NAGLY induces cellular migration through GPR18 by cannabinoid receptor-independent mechanisms (McHugh et al., 2010; McHugh et al., 2012). In mouse macrophage-derived cell line, RAW264.7, NAGLY potently induced GPR18-dependent apoptosis (Takenouchi et al., 2012). In murine anterior eye, NAGLY reported to reduce intraocular pressure independent of cannabinoid CB_1, CB_2 and GPR55 receptors (Caldwell et al., 2013). Recently, Bondarenko et al. (2013) reported inhibitory effect of NAGLY on plasma membrane Na^+–Ca^{2+}exchange (NCX) activity, which largely controls endothelial cell function. NAGLY has also been shown to reversibly hinder store-operated Ca^{2+}entry in a time- and concentration-dependent manner (Deak et al., 2013).

Owing to the unclear vascular activity of NAGLY, its *in vivo* actions and underlying mechanisms of vasorelaxation requires further validation. Hence, the purpose of the current study was to further clarify the

* Corresponding author.
E-mail address: ymksqu@hotmail.com (Y.M. Al Suleimani).

http://dx.doi.org/10.1016/j.ejphar.2016.11.040
Received 18 October 2016; Received in revised form 13 November 2016; Accepted 23 November 2016
Available online 24 November 2016

hemodynamic effects of NAGLY *in vivo* and its vascular actions on isolated arteries. The effect of NAGLY on blood pressure and regional blood flow *in vivo* was examined in rats, and vascular tone was assessed in third order generation of the rat superior mesenteric artery. The roles of the endothelium, nitric oxide, Na^+/Ca^{2+} exchanger and Ca^{2+}-sensitive K^+ channels (K_{Ca}) as well as of the transient receptor potential cation channel subfamily V member 1 ($TRPV_1$) receptors, cannabinoid CB_1 and CB_2 receptors, GPR55, peroxisome proliferator-activated receptor type gamma (PPARγ) and the putative endothelial cannabinoid receptor sensitive to rimonabant and O-1918 were examined. Furthermore, the effect of NAGLY on carbachol- and phenylephrine-induced tone change was also assessed.

2. Materials and methods

Animal procedures were approved by the Animal Ethical Committee of Sultan Qaboos University (SQU) and were in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 85-23, 1985).

2.1. Hemodynamic studies

Male Wistar rats (250–400 g) were obtained from the Animal House of SQU. They were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). PE50 cannulae, filled with heparinized normal saline (25 IU ml^{-1} in 0.9% NaCl), were inserted into the right carotid artery for the measurement of blood pressure by a pressure transducer (TSD104A, Biopac Systems, Santa Barbara, CA, USA), and into the right jugular vein for the administration of drugs. Blood pressure was monitored on an MP 150 data acquisition system (Biopac Systems, Santa Barbara, CA, USA). An ultrasonic probe (1RB, Hughes Sacks Electronik-Harvard Apparatus, March-Hugstetten, Germany) was placed around the left renal artery to measure renal blood flow and was connected to a flow meter (Hughes Sacks Electronik-Harvard Apparatus, March-Hugstetten, Germany).

2.2. Myograph studies

Male Wistar rats (250–400 g) from same source were killed with an overdose of sodium pentobarbital (120 mg/kg, i.p). The mesentery was rapidly removed and placed in ice-cold, gassed (95% O_2/5% CO_2), Krebs-Henseleit solution (pH 7.4) of the following composition (mM): NaCl, 118; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; $CaCl_2$, 2.5; D-glucose, 5.5). Small branches of the superior mesenteric artery were dissected, cleaned of surrounding tissues and cut into 2 mm-long segments. These were mounted in a wire myograph (Danish MyoTechnology, Aarhus, Denmark) maintained at 37 °C in gassed (95% O_2/5% CO_2) Krebs-Henseleit solution.

The arteries were allowed to equilibrate under zero tension for 15–20 min and then were normalized to a tension equivalent to that generated at 90% of the vessel diameter at 100 mmHg (White and Hiley, 1997). After normalization, the vessels were left for another 10–15 min before a test for endothelial integrity which was assessed by submaximal contraction with phenylephrine (10 μM), followed by relaxation with carbachol (10 μM). Vessels were considered to have functional endothelium when carbachol (10 μM) reduced phenylephrine-induced tone by > 90%. If endothelium was not required, it was removed by rubbing the intimal surface with a human hair and vessels which relaxed < 10% to carbachol were designated as endothelium-denuded. Force of contraction was recorded on a PowerLab recording system (ADInstruments) connected to a personal computer.

In order to study the vasorelaxant effects of NAGLY, vessels were precontracted with phenylephrine (10 μM) and once a stable level of tone had been achieved, a cumulative concentration-response curve to NAGLY was constructed.

In order to study the effect of receptor antagonists, channel blockers or enzyme inhibitors, these were incubated in the bathing solution for 30 min before addition of NAGLY, and then were present during the construction of concentration-response curve. In the case of GW9662 (see below), incubation was for 2 h.

In those experiments where the effect of NAGLY on tone change induced by carbachol and phenylephrine was tested, it was incubated for 30 min before construction of concentration-response curves.

Experiments were conducted in a paired fashion, with control and test experiments carried out on arteries from the same animal. Each preparation was only exposed to a single compound unless otherwise mentioned.

2.3. Data and statistical analysis

Data are expressed as percentage change from baseline. Vertical lines represent S.E.M and *n* is the number of rats. The mean relaxation achieved at the highest concentration of NAGLY used ($R_{30\ \mu M}$) is provided.

Concentration-response curves were analysed using two-way ANOVA of the whole dataset. In experiments where the effect of single concentrations of NAGLY was assessed, statistical comparisons between individual groups were carried out using one-way ANOVA followed by Bonferroni's multiple comparison tests. *P* values of < 0.05 were set as criterion of statistical significance. Statistical analyses were performed using GraphPad Prism version 6 (San Diego, CA, USA).

2.4. Drugs

Phenylephrine, carbachol, indomethacin, L-nitroarginine methyl ester (L-NAME; all from the Sigma Chemical Company, Poole, Dorset) and iberiotoxin, (from Tocris Cookson, Bristol) were dissolved in distilled water. N-arachidonoyl glycine (NAGLY), O-1918, AM 251, rimonabant, capsazepine, 1*H* [1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ), TRAM-34 (all from Tocris) and GW9662 (from Santa Cruz Biotechnology, Dallas, Texas) were dissolved in 100% ethanol. AM 630, CID 16020046 (all from Tocris) and KB-R7943 (from Santa Cruz Biotechnology, Dallas, Texas) were dissolved in 100% dimethyl sulphoxide. All compounds were diluted in distilled water and used within a maximum of two days of preparation.

3. Results

3.1. Effect of NAGLY on mean arterial pressure and renal blood flow

A single dose of NAGLY (1 mg/kg, i.v.) produced a rapid and profound decrease in mean arterial pressure (NAGLY: 69.4 ± 5.5%; vehicle: 7.0 ± 3.1%, n =8, $P < 0.001$; Fig. 1). The maximum decrease achieved within 100 s following NAGLY injection and the rate of recovery was relatively slow and it took almost 5 min to go back to the baseline. Similarly, the same dose of NAGLY reduced rat renal blood flow by 88% (n =8; $P < 0.001$; Fig. 1). Pre-treatment with O-1918 (3 mg/kg, i.v.) for 10 min did not affect NAGLY-caused reduction in both mean arterial pressure and renal blood flow (Fig. 1B).

3.2. Effect of endothelial removal on NAGLY-induced vasorelaxation

NAGLY caused concentration-dependent relaxation of phenylephrine-induced tone in endothelium-intact small mesenteric arteries (Fig. 2A, n =5). The same Fig. also shows that NAGLY-induced vasorelaxation was greatly reduced by the removal of endothelium, leaving a residual relaxation of only 9.5 ± 5.0% (30 μM NAGLY, n =6). Fig. 2B illustrates original trace of the vasorelaxation to NAGLY in the rat small mesenteric arteries in the presence of functional endothelium.