2-Arachidonoyl glycerol induces contraction of isolated rat aorta: role of cyclooxygenase-derived products

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Abstract

Objectives: Endocannabinoids have been shown to play a role in the regulation of vascular tone. The effects of 2-arachidonoyl glycerol (2-AG) on induced-tone were examined in rat aortic rings in vitro. Methods: Aortic rings from Wistar Kyoto (WKY) rats were suspended in organ chambers for recording isometric tension development in response to 2-AG. The production of TXA2 in response to 2-AG was also assessed by enzyme immunoassay. Results: In endothelium-intact rings pre-contracted to PGF2α, 2-AG (10 nM–30 μM) induced a biphasic effect: a weak relaxation from 10 nM to 0.1 μM, which turned into a concentration-dependent contraction from 3 to 30 μM. Endothelium-denudation did not change 2-AG-mediated vascular effects. 2-AG-induced contraction was unaffected by both the cannabinoid CB1 receptor antagonist SR141716A (3 μM) and the CB2 receptor antagonist SR144528 (1 μM). In contrast, the anandamine transport inhibitor (AM404, 100 μM) and the amino hydrolase inhibitor (PMSF, 30 μM) attenuated (P<0.05) the contractile response evoked by 2-AG in endothelium-intact and rubbed aortic rings. In addition, the cyclooxygenase inhibitor (indomethacin, 10 μM) and the thromboxane A2 (TXA2) receptor (TP receptor) antagonist GR32191 (0.3 μM) totally abolished the contraction elicited by 2-AG in endothelium-intact and rubbed aortic rings. Challenge of isolated aortic rings with 2-AG (10 μM) evoked a significant increase in TXA2 level (measured as TXB2 level) in endothelium-intact and rubbed aortic rings. Conclusion: These data suggested that the contraction elicited by 2-AG resulted from the vascular smooth muscle cell uptake and conversion of 2-AG to constrictor prostanoid TXA2, which in turn caused vasoconstriction through the stimulation of TP receptor.

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1. Introduction

Endogenous cannabis-like compounds (or “endocannabinoids”) are a small family of naturally occurring lipids that include anandamide (arachidonyl ethanolamide) and 2-arachidonoyl glycerol (2-AG) [1]. It is now well established that cannabinoids elicit not only neurobehavioral but also cardiovascular effects. In particular, the vascular effects of anandamide have been extensively studied. Anandamide produces a vasorelaxation in a wide variety of vascular beds, including cat cerebral artery [2], sheep coronary artery [3], rat mesenteric [4–6] and hepatic artery [7]. In contrast, 2-AG has received less attention, and few studies have investigated its vascular effects. However, 2-AG is an agonist of both cannabinoid CB1 and CB2 receptors and is released by endothelial cells [8]. Therefore, 2-AG may be involved in the local regulation of vascular tone. Indeed, in anaesthetised rats [9] and mice [10], intravenous injection of 2-AG produces a brief hypotension that is prevented by the cyclooxygenase inhibitor indomethacin. In addition, 2-AG induces in vitro a full relaxation of rabbit mesenteric artery via an endothelium-independent mechanism [11].

The aim of the present study was to further assess the vascular effects of 2-AG in isolated rat aorta, and to investigate the contribution of cyclooxygenase-derived products in its vascular effects.
2. Methods

This investigation conforms with the guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Experiments were conducted on 45 adult male Wistar Kyoto (WKY) rats (weight range 300–350 g) from Elevage Janvier (France) housed in climate controlled conditions and provided with standard chow.

Animals were anaesthetised by the intra-peritoneal injection of pentobarbital (50 mg kg\(^{-1}\)). After thoracotomia, the thoracic aorta was excised, transferred to dish filled with Krebs bicarbonate buffer, cleared of periadventitial tissue, and cut into ring segments (3 mm in length). In certain rings, the endothelium was removed by gentle rubbing of the intimal surface with small forceps; in the remaining rings, care was taken not to touch the inner surface of the blood vessels.

2.1. Measurement of isometric tension in rings of aorta

Studies of tension development were performed by the use of a method previously reported [12]. The rings of aorta were initially stretched to a given preload of 1.5 g. After a 60-min equilibration period, experiments were initiated by obtaining in each ring a reference contraction in response to KCl 90 mM. The endothelial function was assessed by testing the relaxant effect of acetylcholine (10 nM–0.1 mM) on aortic rings pre-contracted with phenylephrine (30 nM–0.1 \(\mu M\)). The failure of acetylcholine to elicit relaxation of aortic rings previously subjected to rubbing of the intimal surface was taken as proof of endothelium removal. Subsequently, the rings were allowed to equilibrate for another hour, with the Krebs solution being changed every 15 min. Cumulative concentration–response curves for 2-AG (0.5 log increment, 10 nM–30 \(\mu M\)) were then established either on the basal tone or on phenylephrine-precontracted aortic rings.

To investigate the possible involvement of cannabinoid receptors in 2-AG vascular effects, aortic rings were pre-treated for 30 min with either the CB1 receptor antagonist SR141716A (3 \(\mu M\)) [13] or the CB2 receptor antagonist SR144528 (1 \(\mu M\)) [14].

To determine whether prior cellular uptake and metabolic conversion of 2-AG to arachidonic acid was required for the 2-AG vascular effects, aortic rings were pre-treated for 30 min with the endocannabinoid transport inhibitor AM404 (100 \(\mu M\)) [15] or the amino hydroxylase inhibitor phenylmethylsulphonyl fluoride (PMSF, 30 \(\mu M\)) [16].

To examine the contribution of cyclooxygenase (COX)-derived products in the vascular effects of 2-AG, aortic rings were pre-treated for 30 min with either the preferential COX-1 inhibitor indomethacin (10 \(\mu M\)) [17], the COX-2 inhibitor NS-398 (0.1 \(\mu M\)) [17] or the thromboxane A\(_2\) (TXA\(_2\)) receptor (TP receptor) antagonist GR32191 (0.3 \(\mu M\)) [18].

Lastly, cumulative concentration–response curves for 2-AG (0.5 log increment, 10 nM–30 \(\mu M\)) were performed on aortic rings precontracted with KCl.

Appropriate controls (incubation with vehicles) were run under similar experimental conditions in rings obtained from the same aorta. All experiments were conducted with

![Fig. 1. Effect of SR141716A (3 \(\mu M\)) (a) and SR144528 (1 \(\mu M\)) (b) on concentration–response curves to 2-AG in rat aortic rings with (E+) or without (E–) endothelium. Results are expressed as a percentage of phenylephrine-induced tone and are presented as mean ± S.E.M. of experiments performed on 6 to 8 aortic rings. There was no significantly difference between control and SR141716 A- or SR144528-treated aortic rings.](https://academic.oup.com/cardiovascres/article-abstract/63/1/155/282482)
aluminium foil-covered organ bath to prevent light-induced degradation of the drugs.

2.2. Measurement of TXA2, PGF2α and PGE2 release in rings of rat aorta

Level of TXA2 (measured as level of TXB2), PGF2α and PGE2 released by aortic rings in response to 2-AG was measured as previously described [12]. Briefly, intact and rubbed aortic rings were incubated for 30 min with either 2-AG (0.3 or 10 μM) in the presence of indomethacin (10 μM, 30 min), NS-398 (10 μM, 30 min) or vehicle (30 min, control experiments). The Krebs solution was collected and samples were frozen at −80 °C for later measurement of
TXB₂, PGE₂, and PGE₂ levels. The rings were dried in an oven for measurement of dry weight tissue. TXB₂, PGE₂, and PGE₂ levels were measured by enzyme immunoassay according to the manufacturer’s instructions using reagents purchased from Cayman (Ann Arbor, USA). The detection limits of the assays were 13, 8 and 15 pg ml⁻¹, the EC₅₀ values (50% B0⁻¹) were 40, 39 and 51 pg ml⁻¹ for TXB₂, PGE₂, and PGE₂ and the intra and interassay coefficients of variation were <10% for each eicosanoid measurement. We tested 2-AG (10 μM) for cross reactivity with the anti-TXB₂ serum and found no displacement of the tracer at this concentration of 2-AG.

2.2.1. Drugs

Drugs used and their source were: KCl (Prolabo Normapur), acetylcholine, PGE₂, AM404, (N-(4-hydroxyphenyl)arachidonylamine), PMSF (phenylmethylsulphonyl fluoride) and indomethacin were from Sigma, 2-arachidonoyl glycerol was from Biomol. GR32191 was provide by GlaxoWellcome, N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide (SR141716A) and N-(15)-1,3,3-trimethylbicyclo(2.2.1)-hept-2-endoyl)-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)pyrazole-3-carboxamide (SR144528) were provided by Sanofi, NS-398 (N-(2-cyclohexyloxy-4-nitrophenoxy)methane sulfonamide) was from Cayman. Drugs were kept in hept-2-endoyl)-5-(4-chloro-3-methylphenyl)-1-(4-methyl-(2-cyclohexyloxy-4-nitrophenoxy)methane sulfonamide) was from Cayman. Drugs were kept at −20 °C and freshly dissolved in distilled water to the appropriate concentration expressed as final molar concentration in the organ bath.

2.2.2. Statistical analysis

The vascular effects of 2-AG were expressed as phenylephrine-induced tone. The TXB₂, PGE₂, and PGE₂ data were given as pg/mg dry weight tissue. Results are expressed as mean ± standard error of the mean (S.E.M.) for the specified number of preparations tested. Statistical analyses were performed using analysis of variance (ANOVA) followed by Bonferroni corrected t-test. Individual comparisons were made by Student’s t-test for unpaired data. P values <0.05 were considered significant.

3. Results

2-AG induced no modification of the resting tone in aortic rings with or without endothelium (n=4 for each). In contrast, in phenylephrine-precontracted aortic rings from WKY, 2-AG (10 nM–30 μM) induced a biphasic effect: a weak relaxation from 10 nM to 0.1 μM, which turned into concentration-dependent contraction from 3 μM to 30 μM (Fig. 1). These effects of 2-AG were similar in intact or rubbed aortic rings (Fig. 1).

Pre-treatment with the CB1 receptor antagonist SR141716A (3 μM) or the CB2 receptor antagonist SR144528 (1 μM) induced no significant change of 2-AG-evoked change in vascular tone (Fig. 1).

Pre-treatment with either the 2-AG transport inhibitor AM404 (100 μM) or the aminohydrolase inhibitor PMSF (30 μM) did not significantly change the weak relaxation elicited by 2-AG up to 1 μM, whereas it inhibited the contraction elicited by 2-AG in endothelium-intact and rubbed aortic rings (Fig. 2).

Pre-treatment with the preferential COX-1 inhibitor indomethacin (10 μM), the COX-2 inhibitor NS-398 (10 μM) or the TP receptor antagonist GR32191 (0.3 μM) did not significantly change 2-AG-mediated weak relaxation (Fig. 3).
3. In contrast, indomethacin, NS-398 or GR32191 abolished 2-AG-mediated contraction in endothelium-intact and rubbed aortic rings (Fig. 3).

3.1. Release of PGE₂, PGF₂α and TXA₂ in aortic rings

Challenge of intact aortic rings with 2-AG 10 μM induced respectively a 1.7-, 2.2- and 3-fold significant increase over the control values in PGE₂, PGF₂α and TXB₂ levels (Table 1).

Challenge of rubbed aortic rings with 2-AG (0.3 and 10 μM) did not change PGE₂ level, whereas challenge with 2-AG 10 μM induced a significant increase in TXB₂ and PGF₂α levels over their respective basal values (Table 1).

Both on intact and rubbed aortic rings, pre-treatment with indomethacin significantly abrogated 2-AG (10 μM)-mediated increase in eicosanoid release. In contrast, NS-398 had no influence in 2-AG-evoked PGE₂ and PGF₂α release, whereas it abolished 2-AG-evoked TXB₂ release (Table 1).

4. Discussion

The present study demonstrated that the endogenous cannabinoid 2-AG induces contraction of isolated aorta at least in part through its degradation to the constrictor prostanoids TXA₂.

On phenylephrine-induced tone, 2-AG evoked a biphasic effect: a very weak relaxation, which turned into concentration-dependent contraction from 3 to 30 μM. These 2-AG-mediated vascular effects were insensitive to endothelial denudation (present study), suggesting that vasoconstrictor effects of 2-AG involve stimulation of receptor located at the level of smooth muscle cells. However, pre-treatment with the cannabinoid CB1 (SR141416A) and CB2 (SR144528) receptor antagonists did not change 2-AG-evoked contraction. These data suggest that 2-AG evoked vasoconstriction via a cannabinoid receptor-independent mechanism. After release, anandamide and 2-AG may be eliminated by a two-step mechanism consisting of carrier-mediated transport into cells followed by enzymatic hydrolysis [1]. In particular, 2-AG is rapidly metabolized to yields arachidonic acid [19] and glycerol by monoacylglycerol lipase or fatty acid amine hydrolase [20]. In cerebellar membrane preparation, 2-AG is degraded to arachidonic acid up to 45 min and pre-treatment with the amine hydrolase inhibitor PMSF prevents 2-AG degradation [19]. In the present study, the duration of experiments was about 30 min, suggesting that degradation of 2-AG is likely to occur. Moreover, the findings that 2-AG-evoked contraction was attenuated by both the 2-AG transport inhibitor AM404 and the amine hydrolase inhibitor PMSF suggest that 2-AG is taken up by the vasculature and then metabolised. Some effects of anandamide and 2-AG have been attributed to their metabolism to arachidonic acid and subsequently to cyclooxygenase or cytochrome P450. In
line with this later finding, 2-AG, at a concentration that induced a weak relaxation (0.3 μM), failed to stimulate the release of PGE2. These data clearly excluded the involvement of vasorelaxant prostanooids in the weak relaxation elicited by law doses of 2-AG. In this context, we did not measure the release of 6-ketoPGF1α in response to 2-AG since the involvement of PGI2 in 2-AG-mediated-weak relaxation appeared unlikely. Moreover, the vasorelaxant effect of 2-AG persisted on KCl-precontracted aortic rings (data not shown), suggesting that the weak relaxation might not be mediated through the release of hyperpolarisation factor. In contrast, in anaesthetised rat and mice, 2-AG induced a brief hypotensive effect that was abolished by indomethacin [10], indicating that products of cyclooxygenase mediated this effect. All these discrepancies suggest that there are regional and species differences in the vascular effect of 2-AG as previously reported for anandamide [24] and highlight the need for further studies to better characterise the underlying mechanism of 2-AG-induced dilation.

However, the in vitro contractile effect of 2-AG (present study) occurred for much higher concentrations that those reported on rat aorta (0.4 to 2 ng/g wet weight) [9] suggesting that this endocannabinoid may not be involved in aorta vasoconstriction under physiological conditions. In this regard, the quantification of 2-AG in cardiovascular diseases could be of interest in order determine whether 2-AG could play a role in the control of the vascular tone under pathophysiological conditions associated with increased levels of 2-AG.

References