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 adults 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 thoracotomy, 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 hydrolase inhibitor phenylmethysulphonyl 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

\[\text{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.}\]
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

Fig. 2. Effect of AM404 (100 μM) (a) and PMSF (3 μM) (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 10 aortic rings. There was a significant difference (P < 0.05) between control and AM404- or PMSF-treated aortic rings.

Fig. 3. Effect of indomethacin (10 μM) (a), NS-398 (10 μM) (b) and GR32191 (0.3 μM) (c) 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 a significant difference (P < 0.05) between control and indomethacin-, NS-398- or GR32191-treated aortic rings.
Results are expressed as pg/mg dry weight tissue and are presented 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. 2).

Table 1

Release of PGE2, PGF2α, and TXB2 in response to 2-AG (0.3 and 10 μM) or vehicle (control values) in rat aortic rings in the presence or the absence of indomethacin (10 μM) or NS-398 (10 μM)

<table>
<thead>
<tr>
<th></th>
<th>PGE2</th>
<th>PGF2α</th>
<th>TXB2</th>
</tr>
</thead>
<tbody>
<tr>
<td>With endothelium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>185.49 ± 16.6 (8)</td>
<td>95.13 ± 14.7 (8)</td>
<td>17.68 ± 3.5 (7)</td>
</tr>
<tr>
<td>2-AG 0.3 μM</td>
<td>145.85 ± 21.3 (8)</td>
<td>154.8 ± 40.38 (5)</td>
<td>45.3 ± 22.3 (4)</td>
</tr>
<tr>
<td>2-AG 10 μM</td>
<td>313.56 ± 32.1* (8)</td>
<td>216.67 ± 33.4* (9)</td>
<td>68.7 ± 13.6* (8)</td>
</tr>
<tr>
<td>Indomethacin + 2-AG 0.3 μM</td>
<td>19.8 ± 8.7** (6)</td>
<td>14.01 ± 8.4** (4)</td>
<td>&lt;13** (4)</td>
</tr>
<tr>
<td>Indomethacin + 2-AG 10 μM</td>
<td>24.7 ± 7.7** (7)</td>
<td>30.98 ± 9.9** (7)</td>
<td>&lt;13** (6)</td>
</tr>
<tr>
<td>NS-398 + 2-AG 0.3 μM</td>
<td>288.94 ± 53.2 (4)</td>
<td>189.86 ± 19.8 (4)</td>
<td>&lt;13** (4)</td>
</tr>
<tr>
<td>NS-398 + 2-AG 10 μM</td>
<td>285.40 ± 40.7 (4)</td>
<td>197.86 ± 40.1 (4)</td>
<td>14.7 ± 4.1** (4)</td>
</tr>
</tbody>
</table>

Without endothelium

<table>
<thead>
<tr>
<th></th>
<th>PGE2</th>
<th>PGF2α</th>
<th>TXB2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>363.12 ± 33.5 (7)</td>
<td>224.8 ± 42.6 (7)</td>
<td>20.7 ± 2.6 (10)</td>
</tr>
<tr>
<td>2-AG 0.3 μM</td>
<td>323.1 ± 36.1 (7)</td>
<td>243.8 ± 60.7 (6)</td>
<td>51.0 ± 29.2 (4)</td>
</tr>
<tr>
<td>2-AG 10 μM</td>
<td>352.41 ± 61.5 (9)</td>
<td>313.3 ± 45.3** (8)</td>
<td>67.1 ± 24.3* (11)</td>
</tr>
<tr>
<td>Indomethacin + 2-AG 0.3 μM</td>
<td>19.0 ± 5.1** (4)</td>
<td>&lt;8** (4)</td>
<td>&lt;13** (4)</td>
</tr>
<tr>
<td>Indomethacin + 2-AG 10 μM</td>
<td>67.8 ± 53.9** (5)</td>
<td>66.7 ± 33.4** (7)</td>
<td>&lt;13** (6)</td>
</tr>
<tr>
<td>NS-398 + 2-AG 0.3 μM</td>
<td>288.9 ± 53.2 (4)</td>
<td>264.7 ± 75.3 (4)</td>
<td>16.7 ± 3.7** (4)</td>
</tr>
<tr>
<td>NS-398 + 2-AG 10 μM</td>
<td>343.5 ± 49.1 (4)</td>
<td>249.3 ± 71.1 (4)</td>
<td>15.1 ± 2.8** (4)</td>
</tr>
</tbody>
</table>

Results are expressed as pg/mg dry weight tissue and are presented as mean ± S.E.M.

*P < 0.05 vs. respective vehicle.

**P < 0.05 vs. 2-AG (Student’s t-test for unpaired data).
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 vasocontractor 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 the present study, 2-AG-mediated contraction was inhibited by indomethacin and NS-398, suggesting that 2-AG caused contraction through the release of constrictor cyclooxygenase-derived products. Consistent with this hypothesis, the TP receptor antagonist GR32191 also inhibited 2-AG-evoked contraction. Moreover, as previously reported on HCA-7 culture cell lines [21], challenge of intact aortic rings with 2-AG (10 μM) induces an increase of PGE2, TXB2 and PGF2α levels, suggesting the degradation of 2-AG to PGE2, TXA2 and PGF2α. This release was inhibited by indomethacin indicating that TXA2, PGF2α and PGE2 could derive from the COX-1 pathway. Furthermore, since TXA2, PGF2α as well as PGE2 at high concentration are TP receptor agonists [22], these data suggest that 2-AG could evoke its contractile effect through its degradation to TXA2 or PGF2α or PGE2 which in turn activate TP receptor to produce vasoconstriction. In contrast, in endothelium-denuded rings, the contribution of PGE2 appears unlikely since its level was not increased after challenge with 2-AG 10 μM. However, the COX-2 inhibitor NS-398 inhibited TXB2 release in intact and endothelium-denuded aortic rings but not 2-AG-mediated PGE2 and PGF2α release. These data contrast with the previous report of Kozak et al. [21] who demonstrated on HCA-7 human colon adenocarcinoma cells that 2-AG is preferentially converted into prostanoids of the D-, E- and I-series rather than into TXB2 through a COX-2-dependent pathway. This discrepancy could be explained by the different experimental conditions and tissues and highlight the interest of studies on 2-AG metabolism on various tissues. Nevertheless, the inhibitory effect of NS-398 on 2-AG-mediated TXB2 release is consistent with its inhibitory effect on 2-AG-evoked contraction and suggests that TXA2 derived from the COX-2 pathway account for the constrictor effect of 2-AG both in intact and endothelium-denuded aortic rings. In line with these data, Adeagbo et al. [23] reported that COX-2 could be constitutively expressed in rat aortic endothelial and smooth muscle cells. Furthermore, since the inhibitory effects of AM404, PMSF, indomethacin and GR32191 on 2-AG-mediated contraction were insensitive to endothelium denudation, these data confirm that the cellular uptake and metabolic conversion of 2-AG to TXB2 occurs in vascular smooth muscle cells.

The in vitro contractile effect of 2-AG in rat aorta reported in the present study contrast with the vascular effects of 2-AG previously reported [11]. Indeed, in the presence of indomethacin and the NO synthase inhibitor N5-nitro-L-arginine methyl ester, 2-AG induced a great relaxation on rabbit mesenteric artery that was partially inhibited by the CB1 receptor antagonist SR141716A [11]. These data suggest that 2-AG mediated relaxation of mesenteric arteries through stimulation of cannabinoid CB1 receptor but independently of any vasorelaxant prostanoid release. In rat aortic rings (present study), 2-AG (up to 1 μM) induced a weak relaxation that is modified neither with cannabinoid receptor antagonists, nor with indomethacin or NS-398. 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 prostanoids in the weak relaxation elicited by low 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 PGII 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 of 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