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Original research paper

The endocannabinoid anandamide causes endothelium-dependent vasorelaxation in human mesenteric arteries

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ABSTRACT

The endocannabinoid anandamide (AEA) causes vasorelaxation in animal studies. Although circulating AEA levels are increased in many pathologies, little is known about its vascular effects in humans. The aim of this work was to characterise the effects of AEA in human arteries. Ethical approval was granted to obtain mesenteric arteries from patients (n = 31) undergoing bowel resection. Wire myography was used to probe the effects and mechanisms of action of AEA. RT-PCR was used to confirm the presence of receptor mRNA in human aortic endothelial cells (HAECs) and intracellular signalling proteins were measured using multiplex technology. AEA caused vasorelaxation of precontracted human mesenteric arteries with an R_{max} of ~30%. A synthetic CB_1 agonist (CP55940) caused greater vasorelaxation (R_{max} ~60%) while a CB_2 receptor agonist (HU308) had no effect on vascular tone. AEA-induced vasorelaxation was inhibited by removing the endothelium, inhibition of nitric oxide (NO) synthase, antagonising the CB_1 receptor and antagonising the proposed novel endothelial cannabinoid receptor (CB_e). AEA-induced vasorelaxation was not affected by CB_2 antagonism, by depleting sensory neurotransmitters, or inhibiting cyclooxygenase activity. RT-PCR showed CB_1 but not CB_2 receptors were present in HAECs, and AEA and CP55940 had similar profiles in HAECs (increased phosphorylation of JNK, NFκB, ERK, Akt, p70s6K, STAT3 and STAT5). Post hoc analysis of the data set showed that overweight patients and those taking paracetamol had reduced vasorelaxant responses to AEA. These data show that AEA causes moderate endothelium-dependent, NO-dependent vasorelaxation in human mesenteric arteries via activation of CB_1 receptors.

1. Introduction

The first discovered endogenous cannabinoid agonist, anandamide (AEA) was shown to induce vasorelaxation of rabbit cerebral arterioles in the early nineties [1]. Since then, AEA is one of the most widely studied cannabinoids in the vasculature [2]. Animal studies have shown that the acute vasorelaxant response to AEA is underpinned by several pathways including cannabinoid (CB_1, CB_2 and CB_e (proposed cannabinoid receptor located on the endothelium)) receptor activation, activation of transient receptor potential (TRP) channels, with subsequent endothelium derived hyperpolarising factor (EDHF) and/or nitric oxide (NO) mediated relaxation of vascular smooth muscle [3–5]. The original work of Ellis and colleagues [1] also found that AEA causes vasorelaxation through its metabolism to other vasodilator substances. However, in the rat mesentery, metabolism of AEA appears to limit its vasorelaxant effects [6]. A slowly developing (over 2 h) vasorelaxant response to AEA has also been observed in rat aortae [7], which is inhibited by a peroxisome proliferator-activated receptor gamma (PPARγ) antagonist, endothelium removal, NO synthase and superoxide dismutase inhibition.

Despite the wealth of studies showing that AEA causes acute vasorelaxation of mesenteric arteries in several animal species, the effects of AEA are unknown in human mesenteric arteries. Indeed, investigations into the direct effects of AEA in human vasculature are limited and conflicting. AEA is ineffective as a vasorelaxant in myometrial arteries [8]. However, topical application of AEA causes increased blood flow in the forearm circulation via TRPV1 activation [9]. AEA also causes maximal vasorelaxation of human pulmonary arteries through its metabolism to vasoactive prostanoids and activation of CB_e [10]. Interestingly, we recently showed that the mechanisms of action of another endocannabinoid, 2-arachidonoylglycerol (2-AG), is different in human mesenteric arteries compared to that previously observed in animal mesenteric arteries, suggesting further research is required

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to understand the role and effects of endocannabinoids in human vasculature [11].

Plasma AEA concentrations are reported to be between 0.3–2.5 nmol/L [12], but are raised in patients suffering from diseases that affect the cardiovascular system, for example, in obese patients [13], type-2 diabetics [14], patients with coronary dysfunction [15] and in patients with portal hypertension associated with cirrhosis [16]. Animal studies have shown that increased AEA levels are associated with decreased arterial contractions and enhanced vasorelaxant responses in the mesenteric arteries of biliary cirrhotic rats [17]. Domencali and colleagues [18] also showed that the vasorelaxant response to AEA was enhanced in cirrhotic rats, associated with an increase in CB_1 and TPRV1 receptor expression. However, in obese rats, anandamide-induced relaxation is decreased in resistance arteries, associated with decreased cannabinoid receptor expression and increased anandamide degradation [19]. We have shown that the responses to AEA are reduced in the Zucker diabetic model, which appears to be brought about by enhanced metabolism of these endocannabinoids, including the production of vasoconstrictor metabolites acting at the thromboxane receptor (Wheal et al., under review). Looking at the effects of patient characteristics on vasorelaxant responses to 2-AG, we found that 2-AG responses were reduced in those with heart disease and type 2 diabetes, and in those taking NSAIDS, statins or anti-diabetic medication [11].

In light of this background, we hypothesised that anandamide would cause acute vasorelaxation of human mesenteric arteries and that these responses would be affected by medical conditions. To address this hypothesis, the aims of the study were to assess the potential vasorelaxant effect of AEA in isolated human mesenteric arteries, to investigate the mechanisms of how this might be brought about, and to establish any potential effect of disease state on AEA responses.

2. Methods

2.1. Chemicals

All salts, L-NAME, indomethacin and bradykinin were supplied by Sigma Chemical Co. (Poole, UK). AEA, AM251, AM630, and capsaicin were purchased from Tocris (Bristol, UK). L-NAME and indomethacin were dissolved in PSS solution. AEA, bradykinin and capsaicin were all dissolved in ethanol at 10 mmol/L with further dilutions made in distilled water. AM251, O-1918 and AM630 were dissolved in DMSO at 10 mmol/L with further dilutions made in distilled water.

Ethical approval was granted by the Derbyshire Research Ethics Committee and Derbyshire Hospitals Trust Research and Development to take mesenteric tissue from 31 patients undergoing surgical treatment of bowel carcinoma and inflammatory bowel disorders. Patient characteristics for those who gave access to medical notes are presented in Table 1. Informed written consent was taken according to the Declaration of Helsinki. Mesenteric tissue containing small mesenteric arteries (700 ± 49 μm diameter (mean ± s.e.m)) were collected, dissected free of all connective tissue and perivascular fat, mounted on to a Mulvany Halpern myograph and normalised to 90% of 13.3 kPa in physiological saline solution (PSS) as previously described [11,20]. The endothelial response to a single concentration of bradykinin (10 μmol/L) was tested to ensure endothelial integrity, and only vessels showing >70% relaxation were used (mean response was $84 \pm 1.6\%$). After washout, arteries were contracted with a combination of U46619 and endothelin-1. The average level of contraction of all arteries was 17 ± 1 mN (representing $89 \pm 4\%$ of the maximal response to a high potassium solution in these arteries). When a stable tone was

Table 1
Patient characteristics.

Characteristic	Range	Mean ± SEM
Ethnicity	27 UK white	
Male	21	
Female	6	
Age	32–82	66 ± 2
Weight (kg)	49–122	81 ± 4
BMI (kg/m^2)	17.6–36.7	27.4 ± 1
Smoking habits		
Non smokers	21	
0–10 CPD	3	
10–20 CPD	3	
Drinking habits		
<10 units p/w	17	
10–20 units p/w	8	
>20 units p/w	2	
Operation		
Right Hemicolectomy	7	
Left Hemicolectomy	2	
Sigmoid Colectomy	7	
Anterior Resection	6	
Abdominoperineal Resection	1	
Total colectomy	4	
Reason for surgery		
Cancer	16	
Inflammatory bowel disorder	11	
Dukes Staging		
Dukes A	8	
Dukes B	4	
Dukes C	3	
Dukes D	1	
Systolic Blood Pressure (mm/Hg)	110–172	142 ± 3
Diastolic Blood Pressure (mm/Hg)	65 ± 101	84 ± 2
Diabetic	7	
Heart Disease	22	
Heart Failure	0	
Hypercholesterolemia	13	
Hypertensive	15	
α-1 adrenoceptor antagonist (total)	1	
Alfuzosin	0	
terazocin	1	
ACE Inhibitors (total)	7	
Lisinopril	5	
Ramipril	2	
AT1 receptor antagonists (total)	2	
Losartan	1	
Irbesartan	1	
Beta Blockers (total)	5	
Metoprolol	1	
Atenolol	3	
Propranolol	1	
Calcium channel blocker (total)	2	
Amlodipine	1	
Nifedipine	1	
Lodipine	0	
Digoxin	1	
Diuretics (total)	2	
Furosemide	2	
GTN	3	
Hypoglycaemic Medication (total)	5	
Gliclazide	5	
Metformin	4	
Analgesia Medication (total)	12	
Aspirin	4	
Ibuprofen	1	
Paracetamol	6	
CoCodamol	3	
Tramadol	2	
Statin (total)	14	
Atorvastatin	4	
Simvastatin	9	
Pravastatin	1	
Thiazolidinedione (total)	1	
Pioglitazone	1	