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Cannabinoid and lipid-mediated vasorelaxation in retinal microvasculature

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ABSTRACT

The endocannabinoid system plays a role in regulation of vasoactivity in the peripheral vasculature; however, little is known about its role in regulation of the CNS microvasculature. This study investigated the pharmacology of cannabinoids and cannabimimetic lipids in the retinal microvasculature, a CNS vascular bed that is autoregulated. Vessel diameter (edge detector) and calcium transients (fura-2) were recorded from segments of retinal microvasculature isolated from adult, male Fischer 344 rats. Results showed that abnormal cannabidiol (Abn-CBD), an agonist at the putative endothelial cannabinoid receptor, CB_e, inhibited endothelin 1 (ET-1) induced vasoconstriction in retinal arterioles. These actions of Abn-CBD were independent of CB_1/CB_2 receptors and were not mediated by agonists for GPR55 or affected by nitric oxide synthase (NOS) inhibition. However, the vasorelaxant effects of Abn-CBD were abolished when the endothelium was removed and were inhibited by the small Ca^{2+}-sensitive K channel (SK_{Ca}) blocker, apamin. The effects of the endogenous endocannabinoid metabolite, N-arachidonyl glycine (NAGly), a putative agonist for GPR18, were virtually identical to those of Abn-CBD. GPR18 mRNA and protein were present in the retina, and immunohistochemistry demonstrated that GPR18 was localized to the endothelium of retinal vessels. These findings demonstrate that Abn-CBD and NAGly inhibit ET-1 induced vasoconstriction in retinal arterioles by an endothelium-dependent signaling mechanism that involves SK_{Ca} channels. The endothelial localization of GPR18 suggests that GPR18 could contribute to cannabinoid and lipid-mediated retinal vasoactivity.

1. Introduction

The pharmacological actions of cannabinoids are largely mediated through two G protein coupled receptors (GPCRs), cannabinoid type 1 (CB_1) and cannabinoid type 2 (CB_2) receptors (Howlett et al., 2004; Mackie, 2008a, 2008b; Svizenska et al., 2008; Pacher and Kunos, 2013; Pertwee et al., 2010). CB_1 is found throughout the CNS and periphery, and it is activation of this receptor that mediates the behavioral actions of the *Cannabis* constituent, Δ^9-tetrahydrocannabinol (Matsuda et al., 1990). CB_2 is associated with cells of the immune system (Munro et al., 1993), and is a target for some of the immunomodulatory actions of cannabinoids (Ashton and Glass, 2007; Rom and Persidsky, 2013). The two best characterized endocannabionoids, arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG), mediate many of their actions via activation of both CB_1 and CB_2 (Di Marzo,

Abbreviations: Abn-CBD, abnormal cannabidiol,4-[(1*R*,6*R*)-3-methyl-6 (1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol; ACEA, *N*-(2-Chloroethyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide; AM251, *N*-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide; AM630, 6-Iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl) methanone; BK_{Ca}, Large-conductance calcium-activated potassium channel; CB_1, Cannabinoid receptor 1; CB_2, Cannabinoid receptor 2; CB_e, Abn-CBD sensitive endothelial receptor; ET-1, Endothelin-1; ET_A, ET_B, Endothelial receptor A, Endothelial receptor B; GPR18, G-coupled protein receptor 18; GPR55, G-coupled protein receptor 55; Ibtx, Iberiotoxin; L-NAME, L-NAME hydrochloride, NG-Nitro-L-arginine methyl ester hydrochloride; LPI, l-alpha-lysophosphatidylinositol; NAGly, *N*-Arachidonylglycine, *N*-(1-oxo-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenyl)glycine; NO, Nitric oxide; NOS, Nitric Oxide Synthase; O-1602, 5-Methyl-4-[(1*R*,6*R*)-3-methyl-6-(1-cyclohexen-1-yl]-1,3-benzenediol; SK_{Ca}, Small-conductance calcium-activated potassium channel; WIN55212-2 mesylate, (*R*)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate

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2009). However, more recent evidence suggests that these endocannabinoids, as well as other cannabimimetic lipids and cannabinoids, may activate non-CB_1/CB_2 receptors to produce their pharmacological effects (Di Marzo and De Petrocellis, 2010; Mackie, 2008a, 2008b; Mackie and Stella, 2006; Pertwee et al., 2010). Endocannabinoids can also act on multiple GPCR-independent targets, including ion channels, ion channel receptors and nuclear receptors (Console-Bram et al., 2012). This scenario is further complicated by accumulating evidence that the activity of endocannabinoids, more so than conventional neurotransmitters, may be constitutively regulated by the localized activity of biosynthetic and degradative enzymes that contribute to the diverse lipid responses reported for different cells and tissues (Reviewed in Stanley and O'Sullivan, 2013; Baranowska-Kuczko et al., 2012; Ho and Randall, 2007).

The involvement of non-CB_1/CB_2 targets mediating cannabinoid and endocannabinoid actions has been particularly noted in the peripheral vasculature, where the existence of a novel endothelial receptor (CB_e) sensitive to the behaviourally-inactive phytocannabinoid, cannabidiol, and the cannabidiol analog O-1918, is involved in AEA-mediated hypotension (Zakrzeska et al., 2010; reviewed in Stanley and O'Sullivan, 2013). Consistent with this, both AEA and abnormal cannabidiol (Abn-CBD), another cannabidiol analog, produced vasodilation in isolated pulmonary and mesenteric arteries that was blocked by O-1918 and abrogated by endothelium removal (Baranowska-Kuczko et al., 2012; Ho and Hiley, 2003). This vasodilation persisted in animals genetically lacking CB_1 and CB_2 receptors, confirming the involvement of novel non-CB_1/CB_2 targets (Ho and Hiley, 2003; Járai et al., 1999).

Studies investigating candidate non-CB_1/CB_2 receptor targets mediating the CB_e receptor vasorelaxation initially focused on GPR55, which is activated by both Abn-CBD and the cannabinoid, CP55940 (Kapur et al., 2009). However, the vasoactive actions of Abn-CBD persisted in GPR55 knock-out mice (Johns et al., 2007; Ryberg et al., 2007), arguing against GPR55 as the primary receptor mediating non-CB_1/CB_2 vasorelaxation. The AEA metabolite, N-arachidonyl glycine (NAGly), which is an endogenous agonist at GPR18 in macrophages and cell expression systems (McHugh, 2012; McHugh et al., 2010, 2012a, 2012b), like Abn-CBD, also produces non-CB_1/CB_2 receptor-mediated, endothelium-dependent vasodilation in mesenteric arteries (Parmar and Ho, 2010). The vasorelaxant actions of NAGly in mesenteric vessels, like Abn-CBD, involved a $G_{i/o}$ sensitive signaling pathway and activation of high conductance Ca^{2+}-activated K channel (BK_{Ca}) activity (Parmar and Ho, 2010).

Cannabinoids and endocannabinoids produce vasoactive actions in the eye. In the anterior eye, ingestion of *Cannabis* and individual cannabinoids constituents is associated with vasodilation and hyperemia (Green et al., 1978; reviewed in Green, 1979) and in the retina, cannabinoids produce increases in retinal blood flow in humans (Plange et al., 2007). However, despite the documented presence of an ocular endocannabinoid system, including the endocannabinoids AEA, 2-AG, as well their cognate enzymes and cannabinoid receptors (Straiker A. et al., 1999, Straiker A.J. et al. 1999; Hu et al., 2010); reviewed in Yazulla (2008), the vascular pharmacology of cannabinoids and related lipids has not been extensively examined in the eye. This is particularly so for the retina, an autoregulated CNS microvasculature. The present study examined the vascular pharmacology of the cannabinoid, Abn-CBD, and the AEA metabolite, NAGly, in isolated endothelin-1-constricted retinal arterioles. Additionally, given that NAGly has been proposed to be a putative ligand for GPR18 (Kohno et al., 2006; McHugh et al., 2012a, 2012b; McHugh et al., 2010), a receptor that is extensively expressed in anterior ocular tissues, including cornea and ciliary body (Caldwell et al., 2013), we also examined the localization of this receptor in retinal vasculature.

2. Materials and methods

2.1. Animals

The animal protocols in this study conformed to the guidelines of the Canadian Council on Animal Care (CCAC; Ottawa, ON: vol. 1, 2nd edition, 1993; vol. 2, 1984). The Dalhousie University Committee on Laboratory Animals approved all protocols. Three month old, male Fisher 344 rats were purchased from Charles River Laboratories (St. Constant, QC). Animals were maintained on a 12-h light/dark cycle with free access to food and water in the Animal Care Facility at Dalhousie University.

2.2. Functional studies of retinal arterioles

Retinal arterioles were isolated as follows: Rats were weighed and injected with heparin (i.p. 3000 U/kg) to inhibit blood coagulation 30 min before they were deeply anaesthetized with sodium pentobarbital (i.p. 220 mg/kg). In some experiments, the endothelium was removed with CHAPS. For endothelium denudation, animals were first perfused by intracardiac injection of saline solution that contained 16 units of heparin per ml. This was followed by perfusion with 0.3% CHAPS in saline to denude the endothelium. The eyes were then enucleated and both retinas were removed and placed in a low Mg^{2+}/low Ca^{2+} dissociation buffer. Each retina was cut into several pieces with surgical scissors and triturated gently with a fire-polished pipette (0.3 mm) to dissociate retinal tissue.

For measurements of changes in vessel diameter, aliquots (200 μl) of dissociated retinal tissue containing retinal vessel segments were placed onto laminin-coated experimental glass recording dishes (0.75 ml) and allowed to adhere for 15 min before superfusion (3 ml/min) with a buffer solution of the following composition (mM): 145 NaCl; 5 KCl; 5 D-glucose; 10 HEPES; 1 $MgCl_2$; 2.0 $CaCl_2$ (pH 7.3). All experiments were performed at 37 °C. Isolated adherent retinal arterioles (15–35 μm) were then identified under an inverted light microscope and selected for further measurements of vessel diameter. The vessel diameter was recorded at 120 Hz with a video edge detector (Crescent Electronics, Sandy, UT). Images of the retinal microvasculature were captured with a television camera (model TM-640, Pulnix America) and displayed on a monitor. Data for vessel diameter was collected using AxoScope computer software (Axoscope software, 8.02, Molecular Devices, Sunnyvale, CA).

In cases where vessels were used for simultaneous Ca^{2+} and vessel diameter recordings, vessels were gently fixed to the bottom of the dish using glass pipettes to anchor each end of the isolated vessel segment. To carry out intracellular [Ca^{2+}] measurements, retinal vessels were loaded with fura-2 AM (5 μM) in the dark for 90 min at room temperature and then placed in a 0.75 ml chamber on the stage of an inverted microscope. Vessels were then superfused with buffer as described above. Fura-2 fluorescence was recorded and measured with a PTI (Photon Technology International, Birmingham, NJ) fluorescence system. Fura-2 was alternately excited at 340 and 380 nm, and fluorescence emission was measured at 510 nm with a DeltaRam fluorescence system and Felix software (version 1.4, PTI). Emission was measured at 30 points per second for each excitation wavelength. Emission ratios corresponding to 340/380 nm were converted to Ca^{2+} concentration with an in vitro calibration curve determined with known concentrations of Ca^{2+}. In those vessels where Ca^{2+} and vessel contraction were measured simultaneously, the microscope light was split with a dichroic cube. The dichroic cube directed red light to the television camera plus edge detector and sent the remaining light to the photomultiplier tube for fluorescence measurement.