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Selective Ligands and Cellular Effectors of a G Protein-Coupled Endothelial Cannabinoid Receptor

LÁSZLÓ OFFERTÁLER, FONG-MING MO, SÁNDOR BÁTKAI, JIE LIU, MALCOLM BEGG, RAJ K. RAZDAN, BILLY R. MARTIN, RICHARD D. BUKOSKI, and GEORGE KUNOS

Laboratory of Physiologic Studies, National Institute on Alcoholism and Alcohol Abuse, National Institutes of Health, Bethesda, Maryland (L.O., S.B., J.L., M.B., G.K.); Organix, Inc., Woburn, Massachusetts (R.K.R.); Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, Virginia (B.R.M.); and Cardiovascular Disease Research Program, North Carolina Central University, Durham, North Carolina (R.D.B.)

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ABSTRACT

The cannabinoid analog abnormal cannabidiol [abn-cbd; (−)-4-(3–3,4-*trans*-*p*-menthadien-[1,8]-yl)-olivetol] does not bind to CB_1 or CB_2 receptors, yet it acts as a full agonist in relaxing rat isolated mesenteric artery segments. Vasorelaxation by abn-cbd is endothelium-dependent, pertussis toxin-sensitive, and is inhibited by the BK_{Ca} channel inhibitor charybdotoxin, but not by the nitric-oxide synthase inhibitor N^{ω}-nitro-L-arginine methyl ester or by the vanilloid VR1 receptor antagonist capsazepine. The cannabidiol analog O-1918 does not bind to CB_1 or CB_2 receptors and does not cause vasorelaxation at concentrations up to 30 μM, but it does cause concentration-dependent (1–30 μM) inhibition of the vasorelaxant effects of abn-cbd and anandamide. In anesthetized mice, O-1918 dose-dependently inhibits the hypotensive effect of abn-cbd but not the hypotensive effect of the CB_1 receptor agonist (−)-11-OH-Δ^9-tetrahydrocannabinol dimethylheptyl. In human umbilical vein endothelial cells, abn-cbd induces phosphorylation of p42/44 mitogen-activated protein kinase and protein kinase B/Akt, which is inhibited by O-1918, by pertussis toxin or by phosphatidylinositol 3 (PI3) kinase inhibitors. These findings indicate that abn-cbd is a selective agonist and that O-1918 is a selective, silent antagonist of an endothelial "anandamide receptor", which is distinct from CB_1 or CB_2 receptors and is coupled through G_i/G_o to the PI3 kinase/Akt signaling pathway.

Endocannabinoids are recently identified lipid mediators that act as natural ligands for cannabinoid receptors and elicit biological effects similar to those of plant-derived cannabinoids (Mechoulam et al., 1998). In addition to their well-known neurobehavioral effects, cannabinoids influence a number of physiological functions, including cardiovascular variables (Hillard, 2000; Kunos et al., 2000; Randall et al., 2002). It has long been known that Δ^9-tetrahydrocannabinol (THC), the main psychoactive ingredient of the marijuana plant, can cause long-lasting hypotension in rodents (Vollmer et al., 1974). The endocannabinoid anandamide also causes hypotension in anesthetized rats and mice, which is susceptible to inhibition by CB_1 receptor antagonists (Varga et al., 1995; Lake et al., 1997) and is absent in mice devoid of CB_1 receptors (Jarai et al., 1999; Ledent et al., 1999), clearly implicating CB_1 receptors. Additional findings ruled out a central mechanism for this effect (Varga et al., 1996). Cannabinoids inhibit norepinephrine release via presynaptic CB_1 receptors on postganglionic sympathetic nerves (Ishac et al., 1996), which probably accounts for their bradycardic effect (Kunos et al., 2000; Wagner et al., 2001). However, cannabinoids can decrease blood pressure to levels lower than that achieved by elimination of sympathetic tone (Lake et al., 1997), which points to a direct vasodilator mechanism. Indeed, anandamide and its metabolically stable analog (*R*)-methanandamide cause vasodilation in the coronary (Wagner et al., 2001; Ford et al., 2002) and cerebral vasculatures (Ellis et al., 1995; Gebremedhin et al., 1999; Wagner et al., 2001), as tested in anesthetized animals or in isolated organs.

Surprisingly, anandamide-induced mesenteric vasodilation, although moderately sensitive to inhibition by SR141716A, could be dissociated from CB_1 receptors by its presence in mice deficient in CB_1 or in both CB_1 and CB_2 receptors (Jarai et al., 1999). Furthermore, THC or potent synthetic CB_1 receptor agonists do not cause mesenteric vasodilation (Wagner et al., 1999). Anandamide binds to vanilloid VR_1 receptors with micromolar affinity, and this in-

L.O. and F.-M.M. contributed equally to this work.

ABBREVIATIONS: THC, Δ^9-tetrahydrocannabinol; abn-cbd, (−)-4-(3–3,4-*trans*-*p*-menthadien-[1,8]-yl)-olivetol (abnormal cannabidiol); HUVEC, human umbilical vein endothelial cells; L-NAME, N^{ω}-nitro-L-arginine methyl ester; CGRP, calcitonin gene-related peptide; PI3, phosphatidylinositol 3; MAP, mitogen-activated protein; NO, nitric oxide; SR141716A, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; BK_{Ca}, large-conductance calcium-activated potassium channel.

teraction was shown to result in the release from sensory nerve endings of the potent vasodilator peptide CGRP (Zygmunt et al., 1999). There is evidence that this mechanism may account for the endothelium-independent, SR141716A-insensitive component of the vasodilator effect of anandamide in some (Zygmunt et al., 1999; Mukhopadhyay et al., 2002) but not in other vascular beds (White et al., 2001; Harris et al., 2002). The vasodilator response to anandamide also has an endothelium-dependent component that is sensitive to inhibition by SR141716A (White and Hiley, 1997; Chaytor et al., 1999; Wagner et al., 1999; Mukhopadhyay et al., 2002), insensitive to VR_1 receptor blockade (Grainger and Boachie-Ansah, 2001; Mukhopadhyay et al., 2002), and may (Chaytor et al., 1999) or may not be inhibited by gap junction inhibitors (White et al., 2001; Harris et al., 2002; Mukhopadhyay et al., 2002). The finding that (*R*)-methanandamide elicits a similar effect in the rat mesenteric arterial bed rules out the role of arachidonic acid metabolites, which have been proposed to mediate the effect of anandamide in some other vascular preparations (Pratt et al., 1998; Grainger and Boachie-Ansah, 2001).

Abnormal cannabidiol [(−)-4-(3–3,4-*trans*-*p*-menthadien-[1,8]-yl)-olivetol; abn-cbd], a structural analog of the behaviorally inactive marijuana constituent cannabidiol, has been reported to lack behavioral effects but to cause profound hypotension in dogs (Adams et al., 1977). More recently, we reported that abn-cbd elicits endothelium-dependent, SR141716A-sensitive mesenteric vasodilation that could be inhibited by cannabidiol, and that this effect remains unchanged in mice lacking both CB_1 and CB_2 receptors (Jarai et al., 1999). This has led us to postulate the existence of a novel endothelial "anandamide" receptor for which abnormal cannabidiol is a selective agonist and cannabidiol is an antagonist (Jarai et al., 1999; Wagner et al., 1999). Evidence has been presented that a similar anandamide receptor in the rabbit aortic endothelium is coupled to G_i/G_o and functions via NO release (Mukhopadhyay et al., 2002). In an attempt to further characterize this putative receptor, in the present study we examined the mechanisms by which abn-cbd causes vasorelaxation in small arteries isolated from the rat mesentery, identified a chemically modified analog of cannabidiol as a selective, silent antagonist of this receptor, and identified some of its downstream effectors in cultured vascular endothelial cells.

Materials and Methods

Reagents. Abnormal cannabidiol (Jarai et al., 1999) was synthesized as described previously (Razdan et al., 1974). O-1918 (Fig. 1) was synthesized by adding dry K_2CO_3 to a Wheaton-type pressure tube, followed by a solution of (−)-2-(3–3,4-*trans*-*p*-menthadien-[1,8]-yl)-orcinol in dry acetonitrile. Additional acetonitrile was used as washings, which were transferred to the pressure tube. Subsequently, iodomethane was added, and the mixture was stirred and heated to 70 to 80°C in an oil bath for 16 h. After cooling, the mixture was filtered and washed with fresh acetonitrile. The filtrate was concentrated on a rotary evaporator, and the residue was dissolved in ether. The ether solution was washed with HCl and water, and then it was dried and concentrated on a rotary evaporator. It was purified by flash chromatography. The fractions containing the material were monitored by thin layer chromatography, visualized by phosphomolybdic acid, and concentrated. ^{1}H NMR showed appropriate peaks. For the in vitro experiments, abn-cbd, O-1918, and (−)-11-OH-Δ^9-tetrahydrocannabinol dimethylheptyl (HU-210) were dissolved in ethanol. In the in vivo experiments, these drugs were emulsified using ethanol/Alkamuls EL-620/phosphate-buffered saline (1:1:8) to make a 30 mM stock solution stored at 4°C. All drugs were purchased from Sigma Chemical (St. Louis, MO), except for Alkamuls EL-620 (Rhodia Inc., Cranbury, NJ), pertussis toxin (List Biological Laboratories Inc., Campbell, CA), N^{ω}-nitro-L-arginine methyl ester (L-NAME; Alexis Corporation, San Diego, CA), HU-210 (Tocris Cookson Inc., Ballwin, MO), and charybdotoxin and apamin (Bachem Bioscience, King of Prussia, PA). Antibodies against the phosphorylated and unphosphorylated forms of p42/44 MAP kinase and Akt were from New England Biolabs (Beverly, MA).

Tissue Preparation. The mesenteric artery preparation used in this study has been described recently (Bukoski et al., 2002). Briefly, male Sprague-Dawley rats (200–300 g) obtained from Harlan (Indianapolis, IN) were anesthetized with isoflurane, and the mesentery was removed. Branch II or III segments were isolated under a dissecting microscope, and 2-mm segments were mounted in a wire myograph (Kent Scientific Co., Torrington, CT) using 0.001-inch wires. The preparations were maintained in Krebs-Henseleit buffer (150 mM NaCl, 5.4 mM KCl, 1.17 mM $MgSO_4$, 1.18 mM NaH_2PO_4, 6.0 mM $NaHCO_3$, 1.0 mM $CaCl_2$, 20.0 mM HEPES, 5.5 mM glucose, pH 7.4) equilibrated with 5% CO_2 in O_2. A resting tone of 1.0 g was set, and vessels were precontracted by the presence of 5 μM phenylephrine in the medium. Vasodilator responses were expressed as the percentage of relaxation of the phenylephrine-induced tone. The functional integrity of the endothelium was tested in all preparations by >90% relaxation elicited by 10 μM acetylcholine. Endothelial denudation was achieved by rubbing the inside of the vessel with a mounting wire and was verified by the loss of the relaxing response to 10 μM acetylcholine (<10% residual relaxation). Concentration-response curves were generated by the cumulative addition of an agonist. Antagonists were added 20 min before the agonist and remained in the medium throughout the test period.

Blood Pressure Measurements. Mice were anesthetized with pentobarbital sodium (50 mg/kg i.p.), and the femoral artery and vein were cannulated for monitoring blood pressure and drug injections, respectively. The arterial cannula was connected to a pressure transducer and computerized data acquisition system (IOX-Datanalyst, EMKA Technologies, Arlington, VA).

Cell Culture. Human umbilical vein endothelial cells (HUVEC) were purchased from American Type Culture Collection (Manassas, VA) and were maintained in primary culture as described previously (Liu et al., 2000, 2002). Briefly, cells were plated onto 60-mm^2 wells in plastic culture dishes and maintained in EGM-2 BulletKit (BioWhittaker, Walkersville, MD) containing 5% fetal calf serum at 37°C under an atmosphere of 5% CO_2 in air. Preconfluent cultures (two to five passages) were incubated with vehicle or agonist in the presence or absence of an antagonist, as described below.

Western Blotting. The regular medium was replaced with serum-free M199 medium, and cells were incubated for 16 h before the addition of the agonist abn-cbd or vehicle for 30 min. O-1918 was added to the medium 30 min before the agonist, whereas pretreatment with pertussis toxin (400 ng/ml) was maintained for 30 min. After drug treatment, the cells were lysed by the addition of 20 μl of

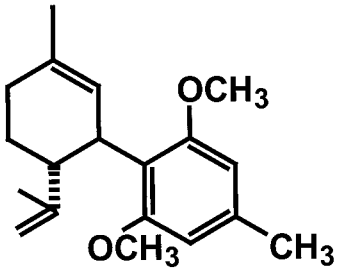

Fig. 1. The chemical structure of O-1918.

radioimmunoprecipitation assay buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.25% sodium deoxycholate, 1 mM phenylmethylsulfonylfluoride, 1 μg/ml aprotinin, 1 mM activated Na_3VO_4, and 1 mM NaF). Aliquots of the cell lysate containing 100 μg of protein were size-fractionated by 10% SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. Phosphorylated and unphosphorylated forms of protein kinase B/Akt and p42/44 MAP kinase were visualized by Western blotting using appropriate antibodies, as described previously (Liu et al., 2002).

Ligand Binding. The ability of abn-cbd and O-1918 to bind to CB_1 or CB_2 receptors was assessed in binding competition assays using [^{3}H]CP-55,940 as the labeled ligand and membranes from mouse cerebellum or from Chinese hamster ovary cells transfected with the human CB_2 receptor cDNA (Showalter et al., 1996), respectively. Details of the binding assays were described previously (Aung et al., 2000).

Statistical Analyses. pA_2 values ($-\log EC_{50}$) were determined in individual concentration-response curves by computerized curve-fitting. Statistical comparisons of concentration-response curves were made by a two-way analysis of variance of the whole data set, followed by the Bonferroni/Dunn post hoc test for determining significant differences ($P < 0.05$) between treatment groups.

Results

Abn-cbd elicits concentration-dependent relaxation of isolated segments of phenylephrine-precontracted mesenteric arteries with a pA_2 of 5.67 ± 0.07 and an E_{max} of 93 ± 3%. The vasorelaxation is gradual in onset and takes 2 to 3 min to reach plateau at each concentration step. Endothelial denudation results in a significant right shift of the abn-cbd dose-response curve (pA_2 = 4.81 ± 0.15; $P < 0.01$) (Fig. 2). HU-210, a synthetic cannabinoid with very high affinity at CB_1 receptors (Lake et al., 1997), is ineffective in relaxing mesenteric artery segments at concentrations up to 1 μM (data not shown). Unexpectedly, cannabidiol, which antagonized abn-cbd–induced vasodilation without causing dilation itself in the rat isolated, buffer-perfused mesenteric vascular bed preparation (Jarai et al., 1999), caused concentration-dependent near-maximal relaxation of isolated arterial segments with a pA_2 value of 5.66 ± 0.06 (data not shown). Therefore, chemically modified analogs of cannabidiol were tested as potential silent antagonists of abn-cbd induced vasorelaxation. The analog O-1918 (Fig. 1) did not induce relaxation at concentrations up to and including 30 μM, but it caused a concentration-dependent right shift of the vasorelaxant effect of abn-cbd (Fig. 3). O-1918 also inhibited vasorelaxation by anandamide (pA_2 = 6.01 ± 0.05 versus 4.59 ± 0.49, $P < 0.05$, in the absence or presence of 10 μM O-1918, respectively).

Abn-cbd and O-1918 were tested for their ability to bind to native CB_1 receptors in mouse cerebellar membranes and cloned mouse CB_2 receptors transfected into Chinese hamster ovary cells. Neither compound displaced the radiolabeled agonist [^{3}H]CP-55,940 from CB_1 or CB_2 receptors at concentrations up to and including 30 μM.

Preincubation of isolated mesenteric arterial segments with 400 ng/ml pertussis toxin for 90 min resulted in a significant right shift of the abn-cbd concentration-response curve in intact but not in endothelium-denuded preparations (Fig. 4). The nitric-oxide synthase inhibitor L-NAME (100 μM) did not affect abn-cbd–induced vasorelaxation (Fig. 5A), suggesting the lack of involvement of endothelial NO. The BK_{Ca} channel inhibitor charybdotoxin caused a significant right shift of the abn-cbd concentration-response curve, which was slightly enhanced in the added presence of the small-conductance calcium-activated potassium channel inhibitor apamin (Fig. 5B). Apamin alone did not significantly affect the response to abn-cbd. The vanilloid VR1 receptor antagonist capsazepine, which inhibits the endothelium-independent component of the vasodilator effect of anandamide (Zygmunt et al., 1999; Mukhopadhyay et al., 2002), did not affect abn-cbd–induced mesenteric vasorelaxation (Fig. 6). At the concentration used (1 μM), capsazepine was an effective antagonist of VR1 receptors, as indicated by its ability to inhibit the vasorelaxant effect of capsaicin (Fig. 6, inset).

In anesthetized mice, the bolus intravenous injection of 10 mg/kg abn-cbd elicited a hypotensive response, which could be dose-dependently inhibited by pretreatment of the animal with 1 to 10 mg/kg O-1918. The higher dose of O-1918 did not

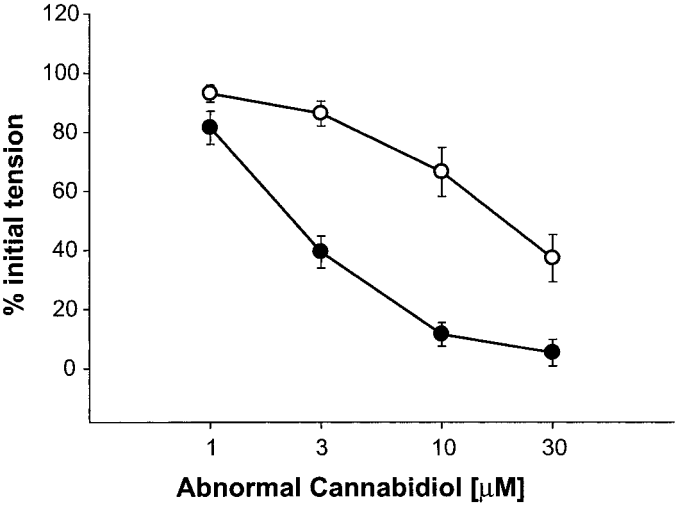

Fig. 2. The vasorelaxant effect of abn-cbd in intact (●) and endothelium-denuded preparations (○). Endothelial denudation was achieved and verified as described under *Materials and Methods.* Each pair of intact and endothelium-denuded preparation was from the same animal. Points and vertical bars represent the means ± S.E. from six pairs of arteries.

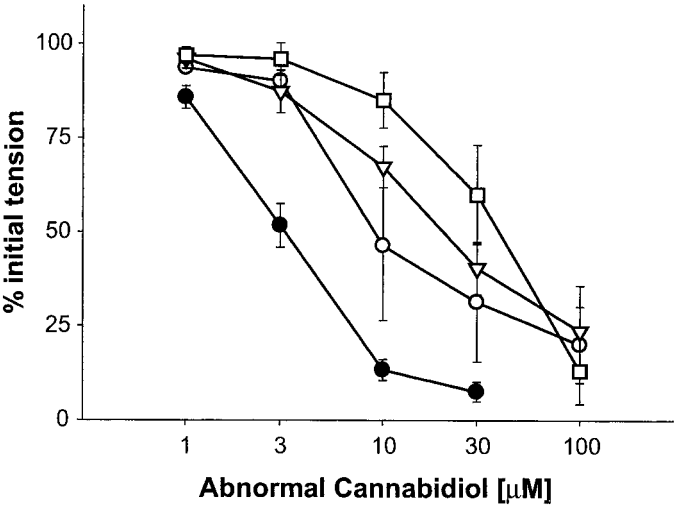

Fig. 3. The vasorelaxant effect of abn-cbd is concentration-dependently inhibited by O-1918 in endothelium-intact mesenteric arteries. The indicated concentrations of abn-cbd were added cumulatively to the tissue bath either alone (●, n = 12), or in the presence of 1 μM (○, n = 4), 10 μM (▽, n = 10), or 30 μM O-1918 (□, n = 5).

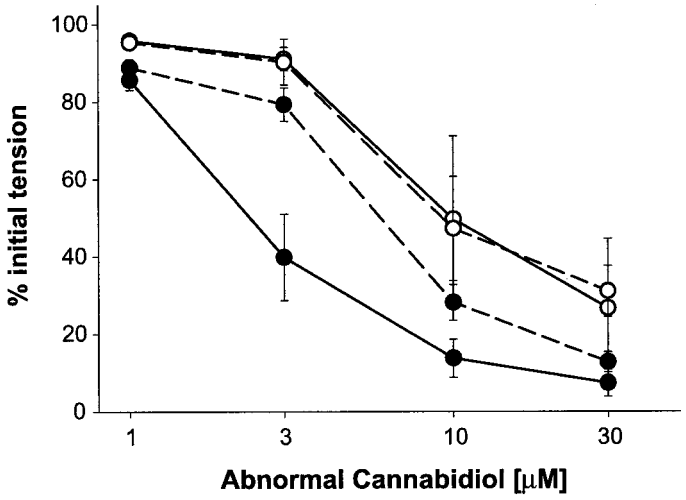

Fig. 4. Pertussis toxin inhibits abn-cbd–induced vasorelaxation in endothelium-intact (●) but not in endothelium-denuded mesenteric arteries (○). Pairs of endothelium-intact or -denuded arteries were preincubated for 90 min with vehicle (solid line) or 400 ng/ml pertussis toxin (broken line) before the cumulative addition of abn-cbd.

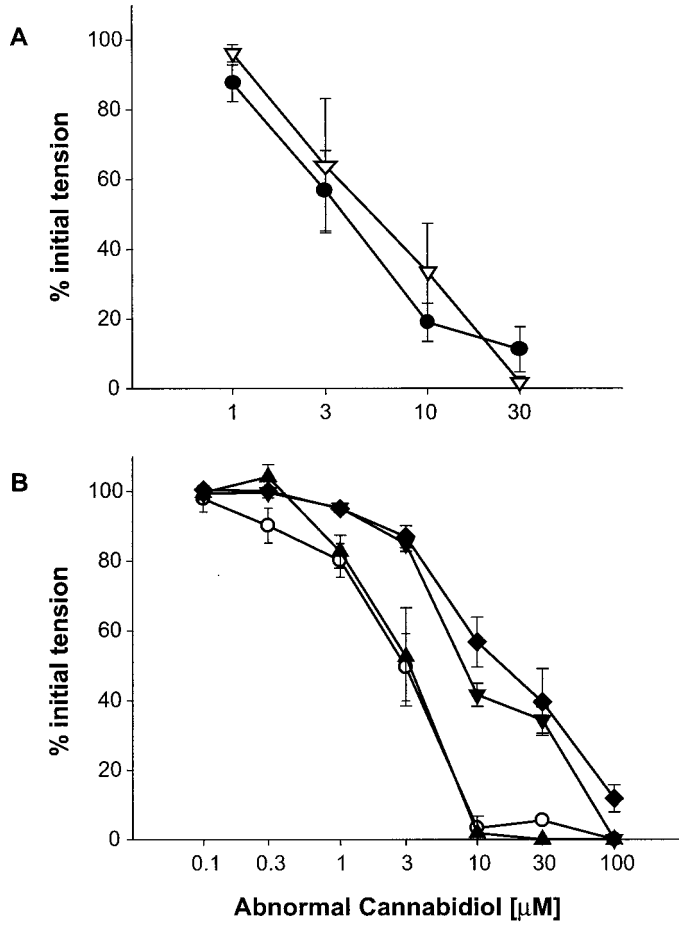

Fig. 5. A, the mesenteric vasorelaxant effect of abn-cbd is independent of endothelial NO. Pairs of arteries were preincubated for 60 min with vehicle or 100 μM L-NAME before the cumulative addition of abn-cbd. B, charybdotoxin, but not apamin, inhibits abn-cbd–induced mesenteric vasorelaxation. Preparations were incubated for 30 min with vehicle (○), 100 nM apamin (▲), 100 nM charybdotoxin (▼), or apamin plus charybdotoxin (♦) before the cumulative addition of abn-cbd.

influence the hypotensive response to subsequently administered HU-210 (Fig. 7), which illustrates the selectivity of the inhibitory effect of O-1918 and confirms its lack of interaction with CB_1 receptors.

Incubation of cultured HUVEC with 10 μM abn-cbd induced rapid phosphorylation of p42/44 MAP kinase and protein kinase B/Akt, which were blocked in the presence of 20 μM O-1918 (Fig. 8). Preincubation of HUVEC with pertussis toxin for 90 min (400 ng/ml) also significantly attenuated the activation of MAP kinase and Akt phosphorylation by abn-cbd (Fig. 8). Protein kinase B/Akt is a downstream effector of PI3 kinase (Cantley, 2002); therefore, we tested whether inhibitors of PI3 kinase can prevent Akt phosphorylation induced by abn-cbd. Both 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (25 μM, Fig. 8) and wortmannin (100 nM; data not shown) blocked Akt phosphorylation induced by abn-cbd in HUVEC.

Discussion

The endogenous cannabinoid anandamide causes vasodilation through both endothelium-dependent and -independent mechanisms, as documented in the rat isolated buffer-perfused mesenteric bed (Jarai et al., 1999; Wagner et al., 1999) and in rabbit isolated aortic rings (Mukhopadhyay et al., 2002), by interacting with sites distinct from CB_1 and CB_2 receptors. The present findings indicate that the synthetic cannabinoid ligands abn-cbd and O-1918 act as a selective agonist and a selective silent antagonist, respectively, of a vascular endothelial receptor that mediates mesenteric vasorelaxation and is coupled to a PI3 kinase/Akt-dependent pathway through G_i/G_o. The finding that O-1918 also inhibits the mesenteric vasorelaxant effect of anandamide strongly suggests that the same endothelial receptor is the site of action of anandamide. That this site is distinct from CB_1 and CB_2 receptors is further indicated by the finding that neither abn-cbd nor O-1918 binds to CB_1 or CB_2 receptors. The

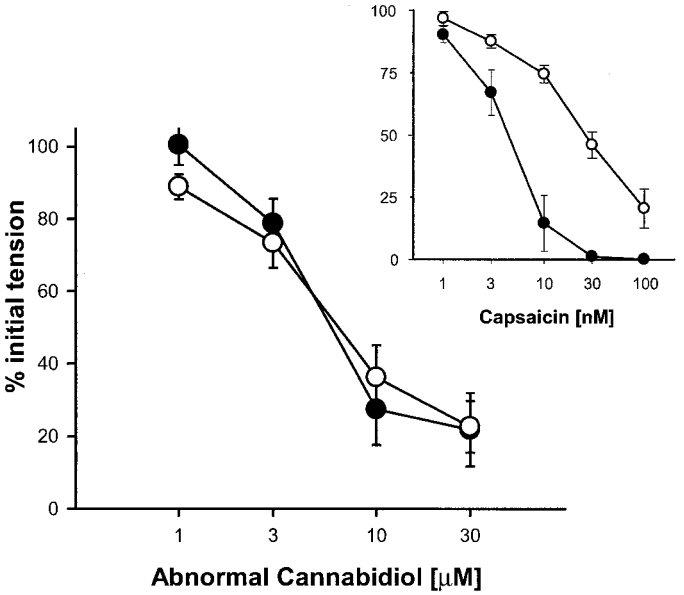

Fig. 6. VR1 receptors are not involved in the mesenteric vasorelaxant effect of abn-cbd. Pairs of arteries were exposed to cumulative concentrations of abn-cbd or capsaicin in the absence (●) or presence (○) of 1 μM capsazepine.

possibility that abn-cbd binds to CB_1 or CB_2 receptors but at a site distinct from the binding sites of conventional antagonists can be also ruled out by the earlier observation that abn-cbd causes mesenteric vasodilation in preparations from CB_1/CB_2 double knockout mice (Jarai et al., 1999).

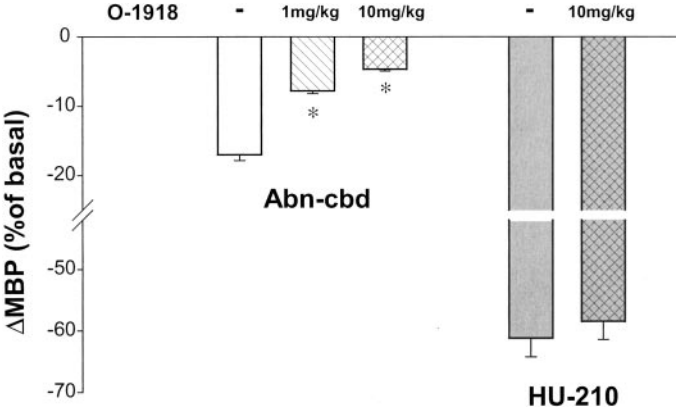

Fig. 7. O-1918 inhibits the hypotensive effect of abn-cbd, but not that of HU-210, in anesthetized mice. Mice anesthetized with sodium pentobarbital were instrumented as described under *Materials and Methods*. Columns and bars represent the means ± S.E. for the peak hypotensive response to 10 mg/kg abn-cbd or 100 μg/kg HU-210 in the absence or presence of the indicated dose of O-1918, injected i.v. 10 min before abn-cbd. Baseline mean blood pressure was 92 ± 6 mm Hg.

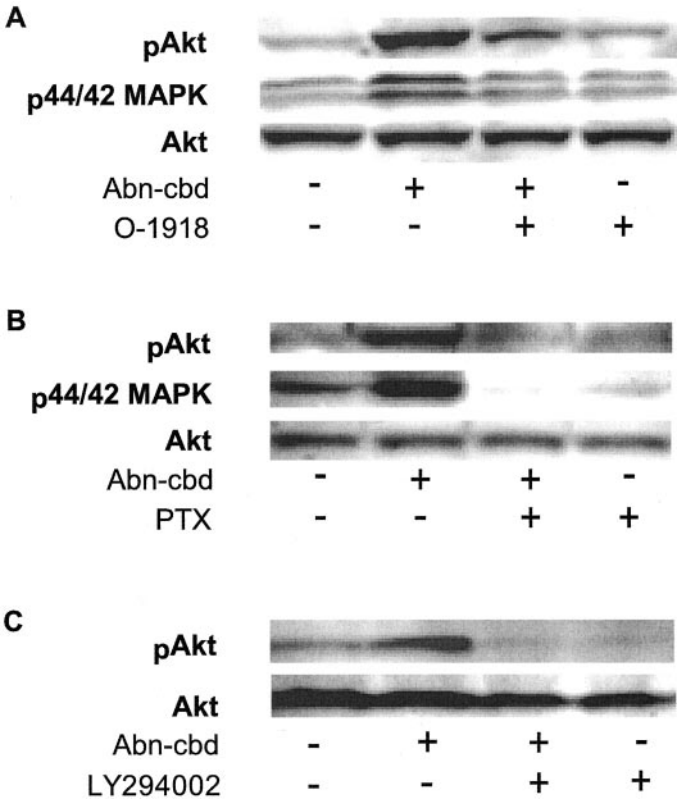

Fig. 8. Abn-cbd induces phosphorylation of p42/44 MAP kinase and protein kinase B/Akt through a PI3 kinase-dependent, G_i/G_o-coupled mechanism in HUVEC. Phosphorylation of Akt and p42/44 MAPK induced by 10 μM abn-cbd is inhibited by 20 μM O-1918 (A) and by 400 ng/ml pertussis toxin (PTX) (B). Akt phosphorylation by 10 μM abn-cbd is inhibited by 25 μM 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002) (C). Western immunoblotting was done using antibodies against pAkt and p42/44 MAPK, as described under *Materials and Methods*. Hybridization with an antibody against the unphosphorylated form of Akt was used to verify equal loading.

The ability of pertussis toxin to inhibit anandamide-induced vasorelaxation was first documented in rat mesenteric arteries (White and Hiley, 1997). In the rabbit isolated aortic ring preparation, anandamide interacts with a non-CB_1/CB_2 endothelial receptor coupled to G_i/G_o and NO synthase, as deduced from the inhibition of its vasorelaxant effect by pertussis toxin and L-NAME, respectively (Mukhopadhyay et al., 2002). In the present experiments, pertussis toxin inhibited the abn-cbd–induced endothelium-dependent vasodilation in rat mesenteric artery segments as well as the activation of p42/44 MAP kinase and Akt phosphorylation in HUVEC, confirming the involvement of a putative G_i/G_o-coupled receptor. However, the NO synthase inhibitor L-NAME did not affect abn-cbd–induced vasorelaxation in rat mesenteric artery segments, which is similar to earlier findings (White and Hiley, 1997; Jarai et al., 1999). In contrast, this effect is inhibited by charybdotoxin, an inhibitor of BK_{Ca} channels as well as delayed rectifier K^+ channels (Garcia et al., 1995). This is similar to previous findings that implicated endothelial K^+ channel activation in the vasorelaxant effect of anandamide (Randall and Kendall, 1997; White and Hiley, 1997; Grainger and Boachie-Ansah, 2001; White et al., 2001), which may be related to endothelium-derived hyperpolarizing factor release (Edwards et al., 1998). It is well established that the relative contribution of NO and endothelium-derived hyperpolarizing factor to endothelially triggered vasodilation varies with the size and location of blood vessels and with the species involved (Hill et al., 2001), and the above discrepancy may reflect such differences.

In an earlier study using the rat isolated buffer-perfused mesenteric bed preparation, the vasodilator action of abn-cbd was fully endothelium-dependent, and cannabidiol acted as a silent antagonist of this effect (Jarai et al., 1999). In the present study, endothelial denudation revealed a residual endothelium-independent component in the dilator action of abn-cbd, and cannabidiol itself caused vasorelaxation, which precluded its testing as an antagonist. It is possible that cannabidiol acts as a silent antagonist in the segment of the mesenteric vasculature that determines the resistance response of the perfused vascular bed preparation, but it does so as a partial agonist in the isolated conduit arteries used in the myograph. Alternatively, intraluminal injection of drugs in the perfused preparation may limit their access to the endothelium, whereas in tissue-bath experiments, they would have equal or even better access to additional sites on smooth muscle and on periarterial nerve terminals. Unlike cannabidiol, its structural analog O-1918 does not cause vasorelaxation but acts as a competitive antagonist of the endothelium-dependent vasodilator effect of abn-cbd and anandamide without antagonizing the response to the muscarinic agonist charbachol. The endothelial site of action of O-1918 is further documented by its ability to antagonize the activation of p42/44 MAP kinase activation and Akt phosphorylation by abn-cbd in HUVEC. Thus, O-1918 is the first selective, silent antagonist of the endothelial anandamide/abn-cbd receptor.

Anandamide binds to vanilloid VR_1 receptors with micromolar affinity, and its mesenteric vasodilator effect has been attributed to a VR_1-mediated release of CGRP from sensory nerve endings (Zygmunt et al., 1999). Indeed, there is evidence that the endothelium-independent (but not the endothelium-dependent) component of the vasorelaxant effect of anandamide is inhibited by the VR_1 antagonist capsazepine

(Mukhopadhyay et al., 2002). In the present experiments using endothelium-intact mesenteric arteries, capsazepine did not affect abn-cbd–induced vasodilation at a concentration that inhibited the effect of capsaicin. This rules out a role for VR_1 in the endothelium-dependent effect of abn-cbd and also indicates that different mechanisms are involved in the endothelium-independent effect of the abn-cbd and anandamide.

Additional cannabinoid receptors distinct from CB_1 and CB_2 may also be present in the central nervous system. In preparations obtained from CB_1 receptor-deficient mice, anandamide and the synthetic cannabinoid agonist WIN55,212–2 have been reported to stimulate guanosine 5′-*O*-(3-thio)triphosphate–labeling in brain plasma membrane preparations (Breivogel et al., 2001) and to inhibit glutamatergic synaptic transmission in hippocampal slices (Hajos et al., 2001). However, these putative receptors are distinct from the vascular endothelial receptor described in the present and previous studies (Jarai et al., 1999; Wagner et al., 1999; Mukhopadhyay et al., 2002). First, these neuronal sites seem to be uniquely sensitive to activation by WIN55,212–2 (Breivogel et al., 2001; Hajos et al., 2001), which was found to be devoid of vasodilator activity in rat mesenteric arteries (Wagner et al., 1999) and in rabbit aortic rings (Mukhopadhyay et al., 2002). Second, our unpublished observations indicate that abn-cbd does not inhibit glutamatergic excitatory postsynaptic potentials in rat hippocampal slices. There is also recent evidence for a THC- or cannabinol-induced, CB_1/CB_2 receptor-independent release of CGRP from sensory nerve terminals (Zygmunt et al., 2002). The inability of THC to induce mesenteric vasorelaxation (Wagner et al., 1999) indicates that this site is also different from the endothelial cannabinoid receptor. Thus, there may be more than one, as-yet-undefined, cannabinoid receptor.

The present observations also indicate that the vasodilator response to activation of abn-cbd–sensitive vascular receptors is sufficient to elicit hypotension in intact animals, at least in the anesthetized state, and that this effect is distinct from CB_1 receptor-mediated hypotension. The question arises as to the possible physiological function of this novel vasodilator mechanism. It has been recently proposed that the mesenteric vasodilator response to increased extracellular Ca^{2+}, such as occurs in the postprandial absorptive phase, is mediated by the release of a sensory nerve-derived endocannabinoid that activates CB_1 receptors, as deduced from the dependence of this response on intact sensory nerves and its susceptibility to inhibition by SR141716A (Ishioka and Bukoski, 1999). Recent evidence that calcium-induced vasorelaxation is maintained in CB_1 receptor knockout mice and is inhibited by O-1918 (Bukoski et al., 2002) suggests that calcium-induced vasodilation is mediated by the receptors described in the present study rather than by CB_1 receptors.

Earlier findings have implicated macrophage-derived endocannabinoids acting at SR141716-sensitive vascular cannabinoid receptors in endotoxic hypotension (Varga et al., 1998). Our unpublished observations that O-1918 inhibits lipopolysaccharide-induced hypotension in mice suggest that the novel endothelial site described here also may be involved in the hypotension associated with septic shock.

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Address correspondence to: Dr. George Kunos, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, 12420 Parklawn Drive, MSC-8115, Bethesda, MD 20892-8115. E-mail: gkunos@mail.nih.gov