G Protein-coupled Endothelial Receptor for Atypical Cannabinoid Ligands Modulates a Ca\(^{2+}\)-dependent K\(^{+}\) Current*

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The cannabinoid analog “abnormal cannabidiol” (abn-cbd) causes endothelium-dependent vasodilation in rat isolated mesenteric arteries through a G protein-coupled receptor distinct from CB\(_1\) or CB\(_2\). We examined the actions of abn-cbd on the electrophysiology of human umbilical vein endothelial cells (HUVEC), using the whole cell version of the patch clamp technique. Voltage steps produced nonactivating outward currents, which were abolished by iberiotoxin or by chelation of intracellular calcium. The presence of a BK\(_{ca}\) channel in HUVEC was documented by reverse transcriptase-PCR. Abn-cbd concentration dependently potentiated the outward current produced by a single voltage step. This potentiation was abolished by the cannabinoid analog O-1918, or by pertussis toxin but was unaffected by CB\(_1\) or CB\(_2\) antagonists. HU-210, a CB\(_1\)/CB\(_2\) receptor agonist, had no effect on the outward current. Clamping [Ca\(^{2+}\)]\(_o\) did not prevent abn-cbd-induced increases in outward current. cGMP potentiated the outward current, and abn-cbd increased the cellular levels of cGMP. The increase in outward current produced by abn-cbd was blocked by KT-5823, an inhibitor of protein kinase G, or 1H-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one (ODQ), an inhibitor of soluble guanylate cyclase. We conclude that a Ca\(^{2+}\)-activated K\(^{+}\) current in HUVEC is potentiated by activation of a G\(_i\)/G\(_o\)-coupled receptor distinct from CB\(_1\) or CB\(_2\), for which signals through cGMP and protein kinase G to increase channel availability or the sensitivity of the channel to voltage and/or Ca\(^{2+}\). Because iberiotoxin also inhibited abn-cbd-induced relaxation of intact, but not of endothelium-denuded, rat mesenteric artery segments, modulation of endothelial BK\(_{ca}\) channels may underlie the mesenteric vasodilator action of abn-cbd.

The marijuana plant contains more than 60 chemical substances of which \(^{\Delta^2}\)tetrahydrocannabinol is the main psychoactive ingredient (1). \(^{\Delta^2}\)Tetrahyrocannabinol produces many of its effects by activating receptors throughout the body. To date, two cannabinoid receptors have been cloned that belong to the superfamily of G\(_i\)/G\(_o\)-protein-coupled receptors. The CB\(_1\) receptor is expressed primarily in the brain (2) but also in peripheral tissues (3), whereas the CB\(_2\) receptor is expressed in immune cells (4). More recently, a number of endogenous cannabinoid ligands have been identified that include, but are not limited to, anandamide and 2-arachidonoyl glycerol (5).

In addition to displaying well described neurobehavioral effects (6), cannabinoid receptor agonists elicit prolonged hypotension and bradycardia (7). These effects are mediated by the CB\(_1\) receptor as shown by the use of the selective CB\(_1\) antagonist SR141716 (8), or CB\(_1\) receptor-deficient mice (9, 10). However, anandamide elicits prolonged vasodilation in the rat isolated mesenteric arterial bed, which, although partially sensitive to blockade by SR141716, is not mimicked by potent CB\(_1\)/CB\(_2\) agonists (11) and persists in mice deficient in CB\(_1\) and CB\(_2\) receptors (10), suggesting the involvement of an as yet undefined site of action.

Abnormal cannabidiol (abn-cbd), a structural analog of the behaviorally inactive marijuana constituent cannabidiol, had been reported to lack behavioral effects but to cause profound hypotension in dogs (12). Abn-cbd elicits endothelium-dependent vasodilation in the isolated mesenteric arterial bed of the rat and mouse, including mice lacking both CB\(_1\) and CB\(_2\) receptors (10). More recently, a study of the responses of isolated segments of rat mesenteric arteries indicated that the endothelium-dependent vasorelaxing effect of both anandamide and abd-cbd could be inhibited by another structural analog of cannabidiol, O-1918 (13), as well as by pertussis toxin (13, 14), and a similar site has been identified in the rabbit aortic endothelium (15). Vanilloid VR1 receptors, which mediate the endothelium-independent component of the vasodilator effect of anandamide (15, 16), do not have a role in this endothelium-dependent vasodilator response (10, 13–15). This has led us to postulate the existence of a novel, endothelial cannabinoid receptor coupled to G\(_i\)/G\(_o\), for which abn-cbd is a selective agonist and O-1918 is a silent antagonist (13).

The mesenteric vasorelaxation produced by abn-cbd is independent of nitric oxide, but it is inhibited by charbydotoxin (13, 14) or iberiotoxin (14), suggesting the involvement of a Ca\(^{2+}\)-activated K\(^{+}\) channel. The involvement of Ca\(^{2+}\)-activated K\(^{+}\) channels in vasodilation has also been identified in rat coronary (17) and mesenteric arteries (18). These latter studies have suggested that cGMP may act as the second messenger in this vasodilatory response either by acting directly on the Ca\(^{2+}\)-activated K\(^{+}\) channel (17) or through the activation of Ca\(^{2+}\).
protein kinase G (18). Ca2+-activated K+ currents in endothelial cells can be studied using the whole cell patch clamp technique (19). In an attempt to clarify the signaling by this putative endothelial cannabinoid receptor and its coupling to ion channels, we investigated the electrophysiological effects of abn-cbd in primary cultured human vascular endothelial cells.

EXPERIMENTAL PROCEDURES

Cell Culture—Human umbilical vein endothelial cells (HUVEC) were from the American Type Culture Collection (Arlington, VA) and maintained in primary culture as described (20). Briefly, the cells were plated onto 35-mm2 plastic culture dishes and maintained in EGM-2 (Lonza, Basel, Switzerland) supplemented with 10% fetal calf serum at 37 °C under an atmosphere of 5% CO2 in air. Plated cells were allowed to adhere for 2 h, at which point the medium was replaced by the extracellular solution (150 mM NaCl, 2.5 mM KCl, 1.0 mM MgCl2, 10 mM HEPES, 10 mM glucose, 2.5 mM CaCl2, adjusted to pH 7.4 with KOH). The cells underwent no more than six passages before use.

Patch Clamp—Glass pipettes were made using TW150F-4 capillaries (World Precision Instruments, Sarasota, FL), backfilled with intracellular solution (ICS; 150 mM KCl, 2.5 mM MgCl2, 10 mM HEPES, 10 mM glucose, adjusted to pH 7.2 with KOH), giving a resistance of 1.5–2 MΩ. Chloride-coated silver wire connected the pipette filling fluid to the patch amplifiers of the patch amplifier system (Axon Instruments, Union City, CA) was mounted on a manipulator and connected through a digital interface (Digidata 1320A, Axon Instruments) to a computer. The software pCLAMP 8.1 (Axon Instruments) was used for data acquisition as well as for off-line data analysis. All of the experiments were performed at room temperature (22 °C). Whole cell patch clamp recordings under voltage clamp were performed after Gigaseal formation (21), followed by disruption of the membrane under the pipette by a suction pulse. The process was monitored by applying a test pulse (5 mV, 5 s, 100 Hz) to the cell, which allowed the analysis of series resistance and enabled optimal capacitive transient cancellation. The cells were clamped at a holding potential of -60 mV, which is close to the resting membrane potential of these cells (19). Voltage steps were applied either as a single +100-mV step from holding or increasing voltage from -60 mV to +90 mV at 10-mV increments. For tail current analysis, a single test pulse of +100 mV was applied followed by increasing hyperpolarizing steps from +30 mV to -170 mV in 10-mV increments. For leak subtraction, four scaled replicas of the voltage step were applied prior to the actual step. Responses to these replicas, which reflect only passive membrane properties, were averaged and subtracted from the actual voltage response.

Reverse Transcripase-PCR—Total cellular RNA was isolated from confluent cultures of HUVEC using the Snap Total RNA isolation kit (Invitrogen). Five μg of total RNA was reverse-transcribed by random priming and incubation with 200 units of Moloney murine leukemia virus transcriptase at 37 °C for 1 h. The resulting single-stranded cDNA (5 μl) was then subjected to 35 cycles of PCR (Robocycler 96, Stratagene) under standard conditions. Briefly, the samples were denatured at 94 °C for 5 min and, after the addition of the polymerase, subjected to 35 cycles of amplification each consisting of 1 min at 94 °C, 1 min at 50 °C, and 1 min at 70 °C, with a 7-min extension at 72 °C during the last cycle. Each PCR mixture (100 μl) contained the cDNA template, 1 μM of the primers, 200 μM of dNTPs, 1.5 μM MgCl2, 10 mM Tris-HCl (pH 9.0 at 25 °C), 50 mM KCl, 0.1% Triton X-100, and 2.5 μM Taq polymerase (Promega). The forward versus reverse primers used to amplify a 119-bp segment of the BK<sub>v</sub> channel mRNA were 5'-GAGGAGATGCTCTGACATCGG-3' and 5'-TGCGATTTTTCACAGCAGCA-3', respectively. The amplicon was sized by electrophoresis on a 2% agarose gel. The amplicon was sized by electrophoresis on a 2% agarose gel. An unprimed amplification reaction using the Snap Total RNA isolation kit (Invitrogen) was applied followed by increasing hyperpolarizing steps from 60 mV to 90 mV at 10-mV increments. For leak subtraction, four scaled replicas of the voltage step were applied prior to the actual step. Responses to these replicas, which reflect only passive membrane properties, were averaged and subtracted from the actual voltage response.

RESULTS

Role of BK<sub>v</sub> Channels in Voltage-activated K<sup>+</sup> Current in HUVEC—Voltage steps applied to HUVEC held at -60 mV produced nonactivating outward currents (Fig. 1a). Tail current analysis performed to determine the nature of the ion carrying this current (Fig. 1, b and c) revealed that its reversal potential (-105 ± 5 mV, n = 6) was similar to the value of -103 mV predicted for K<sup>+</sup> using the Nernst equation. The role of K<sup>+</sup> was further confirmed by the observed shift in the reversal potential to -87 ± 4 mV upon increasing extracellular [K<sup>+</sup>] to 5.0 mM (Fig. 1c; n = 3), which also was similar to the predicted value of -85 mV. Furthermore, these currents were abolished by charybdotoxin (100 nM; data not shown; n = 6) or by iberiotoxin (100 nM; Fig. 1d; n = 6). Although charybdotoxin is a relatively nonselective inhibitor of Ca<sup>2+</sup>-activated K<sup>+</sup> channels and also inhibits delayed rectifying K<sup>+</sup> channels (22), the observed inhibition by the selective BK<sub>v</sub> channel inhibitor iberiotoxin (23) indicated the involvement of BK<sub>v</sub> channels. The expression of BK<sub>v</sub> channel mRNA in HUVEC was also confirmed by reverse transcripase-PCR (Fig. 1e).

Abn-cbd Potentials BK<sub>v</sub> Current in HUVEC—A single voltage step of +100 mV produced an average outward current density of 41 ± 9 pA/pF, which was significantly increased by abnormal cannabidiol (abn-cbd) to 80 ± 10 pA/pF at 10 μM (Fig. 2, a and b; n = 6, p < 0.01). The time required for the maximal increase in current was 54 ± 5 s. Iberiotoxin (100 nM) added to the medium at the peak of the abn-cbd-induced increase in current abolished the current completely (Fig. 2b). Sample traces of currents produced by increasing voltage steps (Fig.,
Fig. 1. Identification of outward current produced by voltage steps in patch-clamped HUVEC. a, sample traces of outward currents produced by a family of voltage steps. b, sample trace of currents produced by tail current protocol. c, current-voltage plot of tail current at 2.5 mV and 5 mV K+. d, outward current produced by a single voltage step and its inhibition of by 100 nM iberiotoxin are illustrated by a sample trace (top) and by the mean currents over a 10 min period from 6 experiments (dotted line, bottom). e, presence of BKCa mRNA in HUVEC documented by reverse transcriptase-PCR.

2c), the voltage-current (Fig. 2d) and voltage-conductance relationships in the presence and absence of abn-cbd (Fig. 2e) are also shown. Abn-cbd produced a significant leftward shift in the conductance-voltage relationship, decreasing the V1/2 from 43 ± 4 to 31 ± 3 mV. The slope values were 78.66 (95% confidence limits of 71.32 and 84.15) for control and 68.67 (60.49–75.88) in the presence of abn-cbd, which also increased the maximal conductance. Despite extensive washing of cells to remove abn-cbd, we did not observe reversal of the increase in current, which is similar to the lack of reversibility of abn-cbd-induced mesenteric artery relaxation, probably related to the highly hydrophobic nature of the compound.

Abn-cbd-sensitive Non-CB1/Non-CB2 Receptor Is Coupled to Gi/Gα—The increase in current produced by 10 μM abn-cbd was prevented by pre-exposure of cells to O-1918 for 2 min (10 μM; Fig. 3a) or to pertussis toxin (PTX) for 2 h (400 ng/ml; Fig. 3b). Fig. 3c illustrates the concentration dependence of the effect of abn-cbd on current density at a +100-mV step and its inhibition by O-1918 and by PTX as tested in six replicate experiments. The effect of abn-cbd remained unchanged in the presence of an antagonist of CB1 (SR141716, 1 μM; Fig. 4; a and c; n = 6) or CB2 receptors (SR144528, 1 μM; Fig. 4c; n = 6). Furthermore, the potent CB1/CB2 receptor agonist HU-210 (100 nM), which has no vasodilator activity in the rat mesentery (11), had no effect on the outward current produced by voltage steps (Fig. 4, b and c; n = 6). None of the compounds examined evoked any currents by themselves on HUVEC held at −60 mV (data not shown), and the ethanol vehicle used did not affect the current produced by voltage steps or the current at the −60 mV holding potential (data not shown; n = 6).

To further analyze the function of BKCa, HUVEC were exposed to the selective BKCa opener NS-1619 (10 μM) (24). NS-1619 alone produced an outward current of 207 ± 20 pA (11 ± 3 pA/pF, n = 4), which was completely blocked in the presence of 100 nM iberiotoxin but was unaffected by 10 μM O-1918 (215 ± 18 pA or 12 ± 4 pA/pF; n = 4). NS-1619 also potentiated the current induced by a 100-mV voltage step from 38 ± 10 to 75 ± 12 pA/pF (p < 0.05, n = 4), and again, this effect was blocked by iberiotoxin but not by O-1918.

The endogenous cannabinoid anandamide also increased the outward current evoked by a single voltage step of +100 mV, although its potency was lower than that of abn-cbd. At 10 μM, anandamide had no effect, but at 100 μM it significantly increased the average outward current density from 39 ± 7 to 74 ± 12 pA/pF (n = 6, p < 0.05). The response to 100 μM anandamide was partially inhibited by O-1918 (10 μM, n = 3) and by PTX (400 ng/ml, n = 3), with the maximal current density being reduced to 58 ± 10 and 55 ± 12 pA/pF, respectively (base-line current density was unaffected by either antagonist alone).

Abn-cbd Modulates BKCa Currents Individually of Changes in [Ca2+]i—Removal of calcium from the extracellular solution significantly reduced the outward current evoked by a +100-mV voltage step to 20 ± 7 pA/pF (n = 6, p < 0.01), which
was not affected by O-1918 (10 μM) and pertussis toxin (400 ng/ml). Columns and bars represent the means ± S.E. from six experiments. Significant differences from control were \( p < 0.05 \) (*) and \( p < 0.01 \) (**).

Addition of the protein kinase G inhibitor KT-5823 (100 nM; Fig. 6d; \( n = 6 \)), a stimulator of soluble guanylyl cyclase (YC-1, 1 μM), and cGMP (100 μM) continued to increase the outward current produced by a 100-mV voltage step back to control levels of 43 ± 7 pA/pF (Fig. 6b; \( n = 6 \)). Similarly, the abn-cbd-induced increase in outward current evoked by a +100-mV voltage step was reversed by KT-5823 (100 nM; Fig. 6d; \( n = 6 \)).

The increase in current was also blocked by the soluble guanylate cyclase inhibitor ODQ (25), with 10 μM abn-cbd increasing the current from 43 ± 7 to 82 ± 9 pA/pF in the absence of ODQ but leaving it unchanged at 40 ± 8 pA/pF in the presence of 1 μM ODQ. YC-1 (1 μM), a stimulator of soluble guanylyl cyclase (26), increased the outward current evoked by a +100-mV voltage step to 84 ± 13 pA/pF (\( n = 6 \), \( p < 0.01 \)), and this increase was insensitive to O-1918 (10 μM; Fig. 6d; \( n = 6 \)).

With the \([\text{Ca}^{2+}]_{i}\) clamped at 250 nM by using a combination of BAPTA (100 μM) and CaCl\(_2\) (6.6 mM) in the ICS, both YC-1 (1 μM) and cGMP (100 μM) continued to increase the outward current evoked by a +100-mV voltage step, to 82 ± 11 and 79 ± 10 pA/pF, respectively (\( n = 6 \), \( p < 0.01 \)). There was no additivity between the effects of cGMP and abn-cbd, with the average current density being 85 ± 10 pA/pF in the simultaneous presence of both compounds (\( n = 6 \); Fig. 6d). Similarly, in the combined presence of YC-1 (1 μM) and abn-cbd (10 μM), the average current density was increased to 84 ± 11 pA/pF (\( n = 6 \); Fig. 6d), showing no additivity.

Because the above findings suggest that the abn-cbd-induced increase in the outward current is mediated via cGMP, we tested the effect of abn-cbd on cGMP levels in HUVEC. As shown in Fig. 7, incubation of the cells with 10 μM abn-cbd for...
4 min resulted in a significant, ~3-fold increase in cGMP levels, which was completely prevented in the additional presence of 100 nM O-1918. As expected, YC-1 also caused a marked, almost 10-fold increase in cellular cGMP levels.

**Role of Endothelial BKCa Channel in Mesenteric Vasodilation by Abn-cbd**—In earlier studies, the endothelium-dependent vasodilator effect of abn-cbd in rat isolated mesenteric arteries was inhibited by charybdotoxin (13, 14) and also by iberiotoxin (14). Because in intact vessels iberiotoxin may act through BKCa channels in smooth muscle rather than endothelium, we tested the effect of iberiotoxin in both intact and endothelium-denuded mesenteric artery preparations. As illustrated in Fig. 8, 100 nM iberiotoxin caused a significant right shift of the abn-cbd concentration-response curves in intact vessels but had no effect on the residual vasorelaxant effect of abn-cbd after endothelial denudation. Thus, in both HUVEC and rat mesenteric arteries, abn-cbd appears to act via endothelial BKCa channels.

**DISCUSSION**

Previous work in the rat isolated mesenteric artery has attributed the vasodilator effect of the atypical cannabinoid ligand abn-cbd to activation of a Gli/Go-coupled endothelial receptor distinct from CB₁ and CB₂ receptors (10, 13). Inhibition of this effect by charybdotoxin (13, 14) suggested the involvement of a calcium-activated K⁺ channel. Using the whole cell patch clamp technique, in the present study we have described a voltage-dependent outward current in HUVEC that is carried by K⁺ ions, as indicated by its reversal potential. The current is blocked not only by charybdotoxin but also by iberiotoxin, strongly suggesting the involvement of the BKCa channel, the presence of which in HUVEC has been confirmed by reverse transcriptase-PCR. The requirement for Ca²⁺ to activate the voltage-induced K⁺ current was confirmed by its marked reduction after chelation of intracellular Ca²⁺ by BAPTA. The BKCa channel is known to be activated by rises in [Ca²⁺]ᵢ, and the currents observed in the present study were similar to those described previously for heterologously expressed BKCa channels (27, 28).
Anandamide acted as a full agonist in the rat isolated mesenteric artery preparation with an EC$_{50}$ comparable with that of abn-cbd (13), suggesting that there may be subtle differences between the rat and human receptors. The low apparent efficacy of anandamide for the human endothelial receptor could suggest the existence of an endogenous ligand(s) other than anandamide.

Removal of the intracellular calcium by BAPTA significantly reduced the voltage-induced outward current, and in cells with [Ca$^{2+}$]$_i$, clamped, the outward K$^+$ current was proportional to [Ca$^{2+}$]$_i$. This indicates that the K$^+$ channel involved is heavily dependent on [Ca$^{2+}$]$_i$, consistent with the Ca$^{2+}$-dependence of the BK$_{ca}$ channel (37). Importantly, whereas the [Ca$^{2+}$]$_i$ was maintained at any given level, the outward current could still be increased by abn-cbd. This suggests that rather than mobilizing intracellular Ca$^{2+}$, the signal transduction pathways activated by abn-cbd directly modulate the BK$_{ca}$ channel. One possibility is that the channel becomes more sensitive to Ca$^{2+}$, thus the same voltage stimulation would evoke a larger outward K$^+$ current. However, the data shown in Fig. 5 indicate that potentiation is decreased at lower [Ca$^{2+}$]$_i$, and thus an increase in channel calcium sensitivity is unlikely or may not be the only mechanism involved. The leftward shift in the voltage-conductance curve in the presence of abn-cbd strongly suggests increased voltage dependence of channel activation by this receptor. Modulation of channel voltage sensitivity has been proposed as the mechanism by which ATP increases the activity of BK$_{ca}$ channels expressed in Xenopus oocytes (38). Additionally, an increase in the available channels or their maximum open probability may account for the ability of abn-cbd to potentiate the current at maximally effective voltage and [Ca$^{2+}$]$_i$ levels.

Recently, it has been proposed that TRPV4 Ca$^{2+}$ entry channels in vascular endothelial cells contribute to the vasorelaxant effect of anandamide via its P450 epoxygenase-dependent metabolites (39). TRPV4 channels are unlikely to be involved in the effects of abn-cbd on the outward current or on vascular tone, because these effects are sensitive to pertussis toxin, whereas TRPV4-mediated calcium entry is not. Furthermore, as discussed above, potentiation of the outward current by abn-cbd persisted in the presence of clamped intracellular calcium.

cGMP has been identified in other preparations as the second messenger involved in the activation of Ca$^{2+}$-activated K$^+$ channels (17). In HUVEC, intracellular cGMP increased the voltage-induced outward current, comparable with the effect of abn-cbd. A similar increase in outward current was also produced by YC-1, an activator of soluble guanylyl cyclase. The increases evoked by abn-cbd and cGMP were inhibited by a protein kinase G inhibitor, KT-5823, and the effect of abn-cbd, but not of cGMP, was also blocked by the guanylyl cyclase inhibitor ODQ. Furthermore, cGMP continued to increase the K$^+$ current under Ca$^{2+}$-clamped conditions, indicating that its action is not due to modulation of [Ca$^{2+}$]$_i$. The effects of abn-cbd, cGMP, and YC-1 on K$^+$ currents were not additive, suggesting that these compounds utilize a common intracellular pathway. Finally, abn-cbd was found to increase cellular cGMP levels, and this effect could be inhibited by O-1918. Together, these data suggest that the novel, G$_i$/G$_o$-coupled receptor activated by abn-cbd is positively coupled to guanylyl cyclase to raise intracellular cGMP, which activates protein kinase G. Both cGMP and protein kinase G have been shown to modulate Ca$^{2+}$-dependent K$^+$ channels (17, 40), and phosphorylation of the Ca$^{2+}$ sensor on the channel by protein kinase G may result in its increased sensitivity to voltage and/or Ca$^{2+}$ and hence an increased K$^+$ current. There is precedent for the activation of

![Endothelial Receptor for Atypical Cannabinoids](46193)

**Fig. 8.** Iberiotoxin inhibits abn-cbd-induced vasorelaxation in intact but not in endothelium-denuded rat mesenteric artery. Isolated segments of rat mesenteric artery were set up in a wire myograph and precontracted with phenylephrine, as described under “Experimental Procedures.” Cumulative concentration-response curves to abn-cbd are shown in intact (solid symbols) and endothelium-denuded preparations (open symbols) in the absence (circles) or presence of 100 nM iberiotoxin (triangles). The points and bars represent the means ± S.E. from three separate experiments.

Although abn-cbd did not elicit a current on its own, it caused a concentration-dependent increase in the voltage-induced K$^+$ current that was sensitive to PTX, suggesting the involvement of a G$_i$/G$_o$-coupled receptor. The increase in K$^+$ current by abn-cbd was unaffected by relevant concentrations of SR141716 or SR144528, which argues against the involvement of CB$_1$ and CB$_2$ receptors, respectively. This is further supported by the lack of effect on K$^+$ currents of HU-210, a potent CB$_1$/CB$_2$ receptor agonist, which is devoid of mesenteric vasodilator activity (11). On the other hand, the abn-cbd-induced increase in K$^+$ current was effectively inhibited by O-1918, a structural analog of cannabidiol, which produced no effect by itself. The finding that the iberiotoxin-sensitive current induced by the selective BK$_{ca}$ opener NS-1619 was unaffected by O-1918 indicates that blockade of the effect of abn-cbd by O-1918 occurs at a site proximal to the channel, most likely at the receptor. This compound as well as PTX similarly inhibited the endothelium-dependent vasorelaxing effect of abn-cbd in the rat isolated mesenteric artery (13). This raises the possibility that activation of BK$_{ca}$ channels is involved in the mesenteric vasodilation mediated by this novel endothelial receptor.

The type of Ca$^{2+}$-activated K$^+$ channel expressed in endothelial cells varies with species and tissue and may also depend on culturing conditions (19). Although BK$_{ca}$ channels are absent from the endothelium in some vascular beds (29–31), their presence has been well documented in others, such as endothelial cells of the human cerebral microvasculature (32) and aorta (33), as well as porcine coronary arteries (36). The present finding that iberiotoxin inhibits abn-cbd-induced vasorelaxation in intact, but not in denuded, rat mesenteric artery segments is similar to observations in rat and porcine coronary arteries (36) and indicates that activation of BK$_{ca}$ channels is involved in the vasodilator response to abn-cbd. This justifies drawing analogies between the effects of abn-cbd in cultured HUVEC and those reported earlier using rat mesenteric artery segments.

The endogenous cannabinoid anandamide also increased the K$^+$ current evoked by a single voltage step. However, its effect was only observed at high concentrations, indicating the low potency of anandamide. The effect of anandamide was partially inhibited by O-1918 or pertussis toxin; thus, part of the effect of anandamide is not mediated by the same pathway as abn-cbd.
guanylyl cyclase by a Gs/cAMP-coupled receptor; in tracheal smooth muscle, muscarinic M2 receptors couple to guanylyl cyclase through a Gs/cAMP-dependent mechanism (41).

Activation of a Ca2+-dependent K+ channel could lead to vasodilation in a number of ways. One possibility is that the outward K+ current hyperpolarizes the endothelial cell membrane, thus increasing the driving force for calcium entry (42, 43), which would then stimulate the formation of vasodilator mediators such as nitric oxide and prostacyclin. However, we (13) as well as others (14) have found that vasorelaxation by abn-cbd in rat mesenteric artery segments was unaffected by the presence of inhibitors of nitric-oxide synthase and cyclooxygenase. Alternatively, the increase in calcium entry may stimulate the formation and subsequent release of an endothelium-derived hyperpolarizing factor, which would then cause vasorelaxation by hyperpolarizing the vascular smooth muscle. Yet another possibility is that the increased K+ current may result in increased K+ concentration in the myoendothelial junction, which may itself act as endothelium-derived hyperpolarizing factor by hyperpolarizing the smooth muscle cell membrane through activation of inward rectifying K+ currents or ouabain-sensitive Na+,K+-ATPase (44). This hyperpolarization would reduce the likelihood of activation of voltage-dependent Ca2+ channels in the muscle membrane, decreasing Ca2+ influx, which would lead to vasodilation (44). Finally, endothelial cell hyperpolarization may be transmitted to the smooth muscle cell through gap junctions, leading to smooth muscle relaxation as described above (44).

Although Ca2+-activated K+ channels appear inactive at resting conditions (19), they can be activated by changes in arteriolar flow (45) or by shear stress (46), thus providing a physiological mechanism by which these channels may be activated. BKCa channels have been also implicated in a vasodilator pathway activated by extracellular calcium (47), and the finding that O-1918 inhibited Ca2+-induced mesenteric vasorelaxation in both wild-type and CB1 receptor-deficient mice has implicated the atypical cannabinoid receptor in this effect (48). The increased expression of BKCa in HUVEC with time in culture was proposed to support the involvement of BKCa in cell proliferation (19). In this regard it is noteworthy that abn-cbd promotes microglial migration in an O-1918 reversible manner (49), and we have observed similar effects in HUVEC that could suggest a role for this receptor in angiogenesis. Further studies are required to explore the physiological functions and establish the molecular identity of this endothelial receptor.

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