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Arachidonylcyclopropylamide increases microglial cell migration through cannabinoid CB_2 and abnormal-cannabidiol-sensitive receptors

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Abstract

Microglial cells, the macrophages of the brain, express low, yet detectable levels of cannabinoid CB_1 receptors, which are known to modulate cell migration. To determine if cannabinoid CB_1 receptors expressed by microglial cells modulate their migration, we assessed whether arachidonylcyclopropylamide (ACPA, an agonist shown to selectively activate CB_1 receptors) affects the migration of BV-2 cells, a mouse microglial cell line. We found that ACPA induced a dose-dependent increase in BV-2 cell migration (EC_{50} = 2.2 nM). This ACPA response was blocked by pertussis toxin pretreatment, suggesting the involvement of $G_{i/o}$ protein-coupled receptors. However, the cannabinoid CB_1 receptor antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide-hydrochloride (SR141716A) did not prevent ACPA-induced BV-2 cell migration. Two antagonists of cannabinoid CB_2 receptors *N*-(1,*S*)-endo-1,3,3-trimethyl bicyclo(2,2,1)heptan-2-yl)-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide (SR144528) and cannabinol, as well as two antagonists of the newly identified "abnormal-cannabidiol-sensitive" (abn-CBD) receptors (O-1918 and cannabidiol) prevented this response. Our results suggest that cannabinoid CB_2 receptors and abn-CBD receptors, rather than cannabinoid CB_1 receptors, regulate microglial cell migration, and that ACPA is a broad cannabinoid receptor agonist.

Keywords: Cannabinoid; Microglial cell; Migration; Inflammation

1. Introduction

Several selective agonists for cannabinoid CB_1 receptors have been described. For example, arachidonylcyclopropylamide (ACPA, Fig. 1, an analogue of anandamide, the prototypical endocannabinoid) inhibits forskolin-stimulated cAMP production in Chinese hamster ovary (CHO) cells transfected with human cannabinoid CB_1 receptors (IC_{50} = 2 nM), while it does not have any effect at concentrations up to 10 μM in CHO cells transfected with human cannabinoid CB_2 receptors (Hillard et al., 1999). However, the selectivity of ACPA should be re-evaluated because additional subtypes of cannabinoid receptors have recently been identified.

Abnormal-cannabidiol-sensitive (abn-CBD) receptors were identified by the group of George Kunos using CB_1/CB_2 receptor knockout mice (Járai et al., 1999). They are $G_{i/o}$-coupled receptors and are expressed by endothelial cells of blood vessels, inducing hypotension when activated (Offertáler et al., 2003). Whether other cell types express this receptor is not well known. Abn-CBD receptors are activated by abnormal cannabidiol (a synthetic cannabinoid agonist) and anandamide (Járai et al., 1999), are antagonized by *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamidehydrochloride (SR141716A) and cannabidiol (when applied at micromolar concentrations), and are not affected by *N*-(1,S)-endo-1,3,3-trimethyl bicyclo(2,2,1)heptan-2-yl)-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide (SR144528) (Járai et al., 1999; Bukoski et al., 2002). A novel silent antagonist of abn-CBD receptors, O-1918, which does not displace the binding of high affinity cannabinoid agonists towards cannabinoid CB_1 and CB_2 receptors, has recently been described (Offertáler

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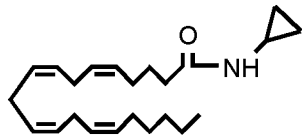

Fig. 1. Structure of arachidonylcyclopropylamide (ACPA).

et al., 2003). The fact that anandamide activates abn-CBD receptors, and ACPA and anandamide are close analogues suggests that ACPA could be an agonist at abn-CBD receptors.

Evidence from our laboratory suggests that microglial cells express cannabinoid CB_1 and CB_2 receptors and abn-CBD receptors (Walter et al., 2003). While we showed that cannabinoid CB_2 and abn-CBD receptors modulate migration of BV-2 cells (Walter et al., 2003), whether CB_1 receptors modulate these cell's migration was not addressed. This is especially relevant considering that cannabinoid CB_1 receptors transfected into human embryonic kidney 293 cells is known to modulate cell migration (Song and Zhong, 2000). Therefore, we sought to (i) determine whether ACPA modulates microglial cell migration, and if so, (ii) characterize the pharmacology of this response.

2. Materials and methods

2.1. Drugs

Pertussis toxin and ACPA were from Calbiochem (San Diego, CA). Cannabinol and cannabidiol were from Sigma (Saint Louis, MO). SR141716A and SR144528 were a gift from Sanofi Research (Montpellier, France). Abn-CBD and O-1918 were a gift from Dr. G. Kunos (National Institute on Drug Abuse and Alcoholism, Bethesda, MD).

2.2. BV-2 cell culture and migration

BV-2 cells, a gift from Dr. E. Blasi (University of Perugia, Perugia, Italy), were expanded in Dulbecco's Modified Eagle Media supplemented with fetal bovine serum (3%), penicillin (100 U/ml) and streptomycin (100 μg/ml) and passaged every 3–4 days for a maximum of 30 passages. For cell migration experiments, BV-2 cells were harvested by using trypsin (0.05%), centrifuged at 200 × *g* for 5 min (to eliminate the trypsin) and re-suspended at 10^6 cells/ml of Modified Eagle Media supplemented with HEPES (10 mM), $NaHCO_3$ (5 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), and CellGro (10%). Cannabinoid compounds were dissolved at 1000 × in dimethyl sulfoxide (DMSO) using siliconized glass vials and added to the lower wells of the Boyden chambers (Wilkinson, 1998) with siliconized pipette tips. BV-2 cells (50 μl) were added to the upper chamber and allowed to migrate through polycarbonate filters (10 μm in pore size) for 3 h at 37 °C (humidified atmosphere of 95% air and 5% CO_2). Lower chamber contained ACPA and antagonists when indicated.

Cells that stayed on the upper surface of the filter were wiped off, whereas cells that had migrated to the undersurface were stained with DIF-Quick stain kit (IMEB). Three scorers that were blinded to experimental conditions manually counted cells in random fields (32 × magnification). We used Prism 2.0 to analyze the data, calculate EC_{50} and draw the dose–response curve.

3. Results

To determine whether cannabinoid CB_1 receptors expressed by BV-2 cells modulate their migration, we used the Boyden chamber assay and quantified BV-2 cell migration towards ACPA. We found that ACPA increased BV-2 cell migration with an EC_{50} of 2.2 nM (Fig. 2). The ACPA response was already significant at 30 nM and maximal at 300 nM (122% over basal migration) (Fig. 2). Whether cannabinoid CB_1 receptors mediate this ACPA-induced BV-2 cell migration was tested with SR141716A, an antagonist at cannabinoid CB_1 receptors (IC_{50} = 6 nM, Rinaldi-Carmona et al., 1994). Surprisingly, 30 nM SR141716A did not prevent the 1 μM ACPA-induced BV-2 cell migration (Fig. 3). Because SR141716A is a competitive antagonist at cannabinoid CB_1 receptors, and ACPA and SR141716A have similar nanomolar affinities towards cannabinoid CB_1 receptors, we assessed whether

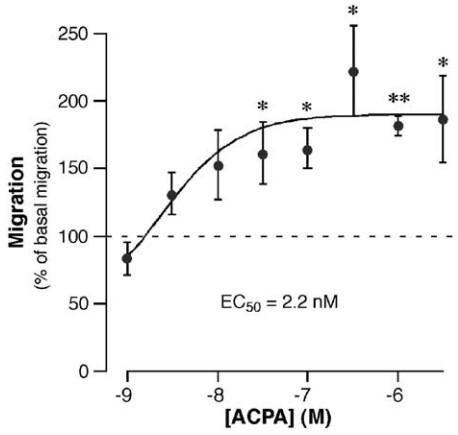

Fig. 2. ACPA increases BV-2 cell migration. Increasing concentrations of ACPA were added in the lower chamber of the Boyden chamber assay and BV-2 cell migration was quantified. Results are expressed in % of basal BV-2 cell migration (i.e., BV-2 cell migration in the presence of vehicle = 0.1% DMSO) measured within individual experiments. Median cells *per* field were 24 cells under basal and 46 cells in the presence of ACPA (1 μM). Under basal conditions, an average of 1.4% of the total cells added in the upper chamber had migrated towards the lower chamber. $^*P<0.05$ and $^{**}P<0.01$ significantly different from basal BV-2 cell migration (ANOVA followed by Dunnett's post-test). Values are mean ± S.E.M. of 9–15 independent quantifications of migration (i.e., three to five experiments performed in triplicate). Dashed line corresponds to control basal migration.

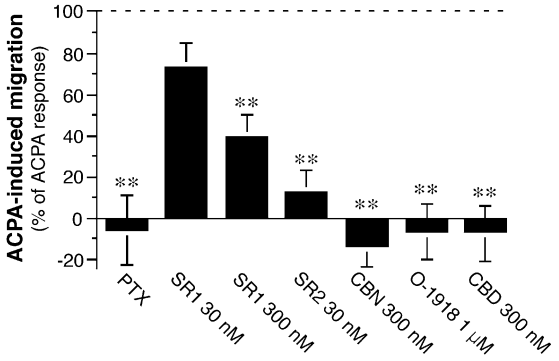

Fig. 3. ACPA increases BV-2 cell migration by acting through cannabinoid CB_2 and abn-CBD receptors. To the lower well of the Boyden chamber, we added ACPA plus SR141716A (SR1), SR144528 (SR2), cannabinol (CBN), cannabidiol (CBD), or O-1918. To test for $G_{i/o}$ protein involvement, BV-2 cells were pretreated 18 h with 1 μg/ml pertussis toxin. Results are expressed in % of the control ACPA-induced migration determined within each experiment (i.e., the difference between cells that migrated in the presence of 1 μM ACPA with antagonist and cells that migrated in the presence of antagonist alone *divided* by the difference between average cell migration induced by 1 μM ACPA and average basal cell migration). Dashed line corresponds to control 1 μM ACPA-induced migration. $*P<0.05$ and $**P<0.01$ significantly different from the control ACPA response within experiment (Student's *t*-test). Values are mean ± S.E.M. of 9–15 independent quantifications of migration (i.e., three to five separate experiments performed in triplicate). Effects of agents on basal BV-2 cell migration were (in % of basal migration): 103 ± 6 for SR1 30 nM, 116 ± 7 for SR1 300 nM, 129 ± 11 for SR2 30 nM, 126 ± 13 for CBN 300 nM, 127 ± 14 for 0-1918 1 μM and 130 ± 10 for CBD 300 nM.

30 nM SR141716A prevents the 100 nM ACPA-induced BV-2 cell migration. However, 30 nM SR141716A only prevented 15% of the 100 nM ACPA-induced BV-2 cell migration ($n=9$, data not shown). Together, these results indicate that ACPA-induced migration occurs through a mechanism independent of cannabinoid CB_1 receptors.

Pertussis toxin pretreatment completely blocked the 1 μM ACPA-induced BV-2 cell migration, suggesting the involvement of $G_{i/o}$ protein-coupled receptors (Fig. 3). We then tested two cannabinoid CB_2 receptor antagonists: SR144528 ($IC_{50}=10$ nM; Rinaldi-Carmona et al., 1998) and cannabinol ($Ki=96$ nM; Showalter et al., 1996). SR144528 (30 nM) and cannabinol (300 nM) both completely prevented the 1 μM ACPA-induced BV-2 cell migration (Fig. 3). Furthermore, the two abn-CBD receptor antagonists, cannabidol (300 nM) and O-1918 (1 μM), also prevented the 1-μM ACPA-induced BV-2 cell migration (Fig. 3). In agreement with the likely involvement of abn-CBD receptors in the ACPA response, 300 nM SR141716A (which, at low micromolar concentrations, antagonizes abn-CBD receptors Járai et al., 1999) prevented 60% of the 1-μM ACPA-induced BV-2 cell migration (Fig. 3).

4. Discussion

Our study shows that ACPA potently increases BV-2 cell migration by acting through cannabinoid CB_2 and abn-CBD receptors. This result brings about several remarks and conclusions. First, cannabinoid CB_1 receptors expressed by BV-2 cells do not seem to modulate their migration. This could be explained by the fact that the majority of cannabinoid CB_1 receptors are located in the intracellular compartment of these cells (Walter et al., 2003). Second, ACPA is not, as previously thought, a selective agonist at cannabinoid CB_1 receptors. Indeed, two cannabinoid CB_2 receptor antagonists, namely SR144528 and cannabinol, antagonized the ACPA-induced BV-2 cell migration. This result is in sharp contrast with the study carried out by Hillard et al., which shows that ACPA merely displaces the binding of [^{3}H] CP55940 towards spleen mouse cannabinoid CB_2 receptors ($Ki=700$ nM) and does not inhibit the forskolin-stimulated cAMP production in CHO cells tranfected with human cannabinoid CB_2 receptors (Hillard et al., 1999). The reason for this discrepancy is unknown at present. Third, ACPA is likely to activate abn-CBD receptors. This result is supported by the structural analogy between ACPA and anandamide, the latter being an agonist at abn-CBD receptors. The molecular cloning of abn-CBD receptors and its pharmacological characterization in heterologous expression systems will further ascertain that ACPA is an agonist at abn-CBD receptors. Finally, ACPA-induced BV-2 cell migration is completely antagonized by cannabinoid CB_2 or abn-CBD receptor antagonists, reinforcing the notion that cannabinoid-induced microglial cell migration requires engagement of both these receptors (Walter et al., 2003). Note that this result agrees with the fact that $G_{i/o}$-coupled cannabinoid CB_2 receptors are present on microspikes of BV-2 cell, cellular protrusions containing polymerized actin filaments that mediate cell migration (Walter et al., 2003; Mitchison and Cramer, 1996; Watanabe and Mitchison, 2002). Although the molecular basis of the synergistic response between cannabinoid CB_2 and abn-CBD receptors remains unknown, selective agonists and/or antagonists at these receptors should provide valuable pharmacological tools to assess the involvement of microglial cell migration in the propagation of neuroinflammation.

Acknowledgements

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