

CB1 Cannabinoid Receptor-Mediated Cell Migration¹

ZHAO-HUI SONG and MIAO ZHONG

Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, Kentucky

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ABSTRACT

Recent studies have suggested that cell migratory responses are often mediated by G_i protein-coupled receptors. Because it is known that CB1 cannabinoid receptors are coupled to pertussis toxin-sensitive G proteins, we proposed that CB1 may mediate cell migration. To test this hypothesis, modified Boyden chamber assays were used to investigate cell migration mediated by CB1 cannabinoid receptors. HU-210, WIN55212-2, and anandamide, three cannabinoid agonists with distinct chemical structures, induced migration of human embryonic kidney 293 cells stably transfected with human *CB1* gene, but not 293 cells transfected with an empty expression vector. These migratory responses were concentration-dependent. The EC₅₀ values for HU-210, WIN55212-2, and anandamide were 0.19 ± 0.04 , 12.2 ± 1.4 , and 39.9 ± 3.7 nM, respectively. The maximal migration index for HU-210, WIN55212-2, and anandamide were 8.9 ± 1.6 , 9.5 ± 1.6 , and 8.8 ± 1.3 , respectively. Pretreating cells with 100 ng/ml per-

tussis toxin eliminated the cannabinoid agonist-induced cell migration. SR141716A, a selective antagonist for CB1, inhibited the cannabinoid agonist-induced migratory responses in a concentration-dependent manner. Checkerboard analysis demonstrated that anandamide-induced cell migrations are due to chemotaxis as well as chemokinesis. Furthermore, anandamide-induced migratory responses were inhibited, in a concentration-dependent manner, by PD098059, an inhibitor of mitogen-activated protein kinase activation, but not by 8-bromo-adenosine-3',5'-cyclic monophosphate, a cell-permeable cAMP analog. These data demonstrate that cannabinoid agonists are able to induce chemotaxis and chemokinesis, and that these migratory responses are mediated by G protein-coupled, CB1 cannabinoid receptors. In addition, these data suggest that activation of mitogen-activated protein kinase plays an important role, whereas inhibition of adenylyl cyclase is probably not involved in the cell migration mediated by CB1.

Since the initial description that Δ^9 -tetrahydrocannabinol, the active constituent of marijuana, interacts with a specific G protein-coupled receptor (Howlett et al., 1986; Devane et al., 1988), two subtypes of cannabinoid receptors, known as CB1 and CB2, have been cloned and identified (Matsuda et al., 1990; Munro et al., 1993). CB1 is distributed in the central nervous system (Herkenham et al., 1991; Matsuda et al., 1993), as well as several peripheral tissues, including testis and immune cells (Gerard et al., 1991; Bouaboula et al., 1993). Using cells that express native cannabinoid receptors and cell lines transfected with cloned cannabinoid receptors, previous studies have demonstrated that CB1 is coupled to pertussis toxin-sensitive G proteins (Howlett et al., 1986; Felder et al., 1992). The known downstream signal transduction pathways of CB1 include inhibition of adenylyl cyclase (Howlett et al., 1986; Felder et al., 1992), activation of mitogen-activated protein kinase (MAPK) (Bouaboula et al., 1995), and modulation of ion channels (Mackie and Hille, 1992).

Cell migration plays important roles in many physiological and pathological processes, including embryogenesis, angiogenesis, metastasis, inflammation, and wound healing (Lauffenburger and Horwitz, 1996). Many chemoattractants are ligands for G protein-coupled receptors. Pertussis toxin-sensitive G proteins are thought to be crucial for the cell migration mediated by chemokine receptors (Gerard and Gerard, 1996). Recent studies have suggested that the ability to mediate cell migration may be shared by many receptors that are coupled to G_i proteins (Arai et al., 1997; Neptune and Bourne, 1997). Because it is known that CB1 is coupled to pertussis toxin-sensitive G proteins, we postulated CB1 may mediate cell migratory responses. To test this hypothesis, the abilities of cannabinoid agonists to induce cell migration were examined in human embryonic kidney 293 cells stably transfected with human *CB1* gene. Cell migration can be classified into three categories: 1) random, 2) chemokinesis, and 3) chemotaxis (Lauffenburger and Horwitz, 1996; Mitchison and Cramer, 1996). Random migrations of cells occur in the absence of a stimulus. Chemokinesis is random motion that is affected by a chemical stimulus. Chemotaxis is directed motion of cells toward a gradient of a chemical stimulus. In this study, checkerboard analysis was per-

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ABBREVIATIONS: MAPK, mitogen-activated protein kinase; 8-Br-cAMP, 8-bromo-adenosine-3',5'-cyclic monophosphate; DMEM, Dulbecco's modified Eagle's medium; ERK, extracellular signal-regulated kinase.

formed to study whether the cannabinoid agonist-induced cell migration is due to either chemokinesis or chemotaxis. Currently, the cellular and molecular mechanisms for cell migration have not been completely understood. It is generally believed that G_i proteins are important in mediating cell migration (Gerard and Gerard, 1996; Arai et al., 1997; Neptune and Bourne, 1997). However, it is not clear which downstream targets of G_i proteins are crucial in cell migration. In this study, the involvement of G protein-coupled CB1 in cell migration was examined with pertussis toxin and with a selective CB1 antagonist. Furthermore, the roles of adenylyl cyclase inhibition and MAPK activation, two known signal transduction pathways for CB1, were investigated using a cell-permeable analog of cAMP and an inhibitor of MAPK activation.

Experimental Procedures

Materials. Adenovirus-transformed 293 cells were obtained from American Type Culture Collection (Rockville, MD). Tissue culture reagents were purchased from Biowhittaker (Walkersville, MD). WIN55212-2, anandamide, and SR141716A were obtained from RBI (Natick, MA). HU-210 was purchased from Tocris (Balwin, MO). The stock solutions of cannabinoids were made by dissolving cannabinoids in dimethyl sulfoxide. BSA, mouse collagen type I, pertussis toxin, 8-bromoadenosine 3',5'-cyclic monophosphate (8-Br-cAMP), and PD098059 were purchased from Sigma (St. Louis, MO). The 48-well modified Boyden chamber and polycarbonate membranes with 10- μ m pore size were purchased from Neuro Probe Inc. (Bethesda, MD).

Cell Culture. Human embryonic kidney 293 cells stably transfected with human *CB1* gene were used (Song and Bonner, 1996). The CB1 cannabinoid receptors expressed in these cells have a $B_{\max}$ value of 1217.6 ± 221.9 fmol/mg of protein. It has been shown that these cells do not express CB2, and the CB1 expressed in these cells are functional (Song and Bonner, 1996). The 293 cell system is an appropriate model system for studying the roles of G protein-coupled receptors in cell migration. This has been shown by other investigators with known inducers of migration, e.g., interleukin-8 receptors (Neptune and Bourne, 1997). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 20 mM L-glutamine. Cells were grown at 37°C in a humidified 5% CO₂ incubator.

Cell Migration Assays. In vitro cell migration assays were performed using a modified 48-well Boyden chamber as described previously (Falk et al., 1980). Briefly, cells were washed twice with PBS and harvested from the culture dish into a 50-ml centrifuge tube with EDTA-trypsin (Biowhittaker). Subsequently, the cells were washed with DMEM supplemented with 0.2% BSA, centrifuged at 500g, and cell pellets were resuspended in the same medium with a final cell concentration of 1.0×10^6 cell/ml. Chemoattractant solutions were made by diluting cannabinoid agonists in DMEM supplemented with 0.2% BSA. Polycarbonate membranes (polyvinylpyrrolidone-free, pore size 10.0 μ m) were coated overnight at 4°C with 50 μ g/ml mouse collagen type I. After loading chemoattractant solutions in the lower chamber, a sheet of polycarbonate membrane was placed on top of the chemoattractant solution, the upper chamber was properly assembled, and aliquots of 50- μ l cell suspensions were placed in the upper chamber. The chamber was incubated for 5 h in a 37°C incubator with humidified air and 5% CO₂. At the end of the incubation period, the polycarbonate membrane was removed, non-migrated cells were carefully removed by scraping against a wiper, and the membrane was stained with a HEMA 3 staining kit (Fisher Scientific Inc., Houston, TX). For each well in the Boyden chamber, the number of cells migrating through to the underside of the mem-

brane was counted in six nonoverlapping low power field ($\times 100$) using a light microscope equipped with an ocular micrometer. The results were expressed as a migration index, which was defined as: mean number of cells per low power field for test substances/mean number of cells per low power field for medium control (DMEM supplemented with 0.2% BSA). Experiments were performed in triplicate and were repeated three times.

Checkerboard Analysis. Chemical-induced increases in cell mobility could be attributed to either chemotaxis or chemokinesis. To distinguish chemotaxis from chemokinesis, checkerboard assays were performed (Zigmond and Hirsch, 1973; Wilkinson, 1998). Briefly, various concentrations of anandamide, an endogenous cannabinoid agonist, were placed in upper wells, lower wells, or both upper and lower wells of the chemotaxis chamber to determine whether the number of migrated cells was greater with a positive gradient, no gradient, or a negative gradient of anandamide. According to the definition of chemotaxis and chemokinesis, cell migration toward a positive gradient represents chemotaxis, whereas drug-stimulated random cell migration (toward no gradient or a negative gradient) represents chemokinesis.

Cell Pretreatment. To study the involvement of pertussis toxin-sensitive G proteins, cells were pretreated for 16 h with 100 ng/ml pertussis toxin in DMEM medium containing 10% fetal calf serum, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 20 mM L-glutamine at 37°C, 95% air, 5% CO₂. The cells were washed and resuspended after pertussis toxin treatment. To study the effects of CB1 antagonist SR141716A, cell-permeable cAMP analog 8-Br-cAMP, and MAPK activation inhibitor PD098059, cell suspensions were pretreated with these agents for 30 min in DMEM supplemented with 0.2% BSA before being subjected to cell migration assays.

Data Analysis. The data presented in the table and figures represent mean $\pm$ S.E. The data were analyzed and the concentration-response curves were generated with the use of GraphPad Prism software. The EC₅₀ and maximal migration index values were determined through nonlinear regression analysis performed with GraphPad Prism. One-way or two-way ANOVA was used to compare the data of different treatment groups. The level of significance was chosen as $P < .05$.

Results

Effects of Cannabinoid Agonists on Cell Migration. HU-210, WIN55212-2, and anandamide, three cannabinoid agonists that belong to three different chemical classes (Mechoulam et al., 1988; Compton et al., 1992; Devane et al., 1992), increased the migration of 293 cells stably transfected with human *CB1* gene from the upper chamber to the underside of the membrane (Fig. 1). The effects of cannabinoid agonists on cell migration were concentration-dependent ($P < .05$ by ANOVA). The EC₅₀ values for HU-210, WIN55212-2, and anandamide were 0.19 ± 0.04 , 12.2 ± 1.4 , and 39.9 ± 3.7 nM, respectively. Approximately 30 to 50 cells migrated in the absence of drugs. The maximal migration index values for HU-210, WIN55212-2, and anandamide were 8.9 ± 1.6 , 9.5 ± 1.6 , and 8.8 ± 1.3 , respectively. In contrast, cannabinoid agonists did not affect the migration of 293 cells transfected with an empty RC/CMV expression ($P > .05$ by ANOVA) (Fig. 1).

Effects of Pertussis Toxin on Cannabinoid Agonist-Induced Cell Migration. To study the involvement of pertussis toxin-sensitive G proteins in cannabinoid agonist-induced cell migration, cells were pretreated with 100 ng/ml pertussis toxin for 16 h. As demonstrated in Fig. 1, pretreatment of cells with pertussis toxin blocked the cell migration induced by HU-210, WIN55212-2, and anandamide ($P < .05$

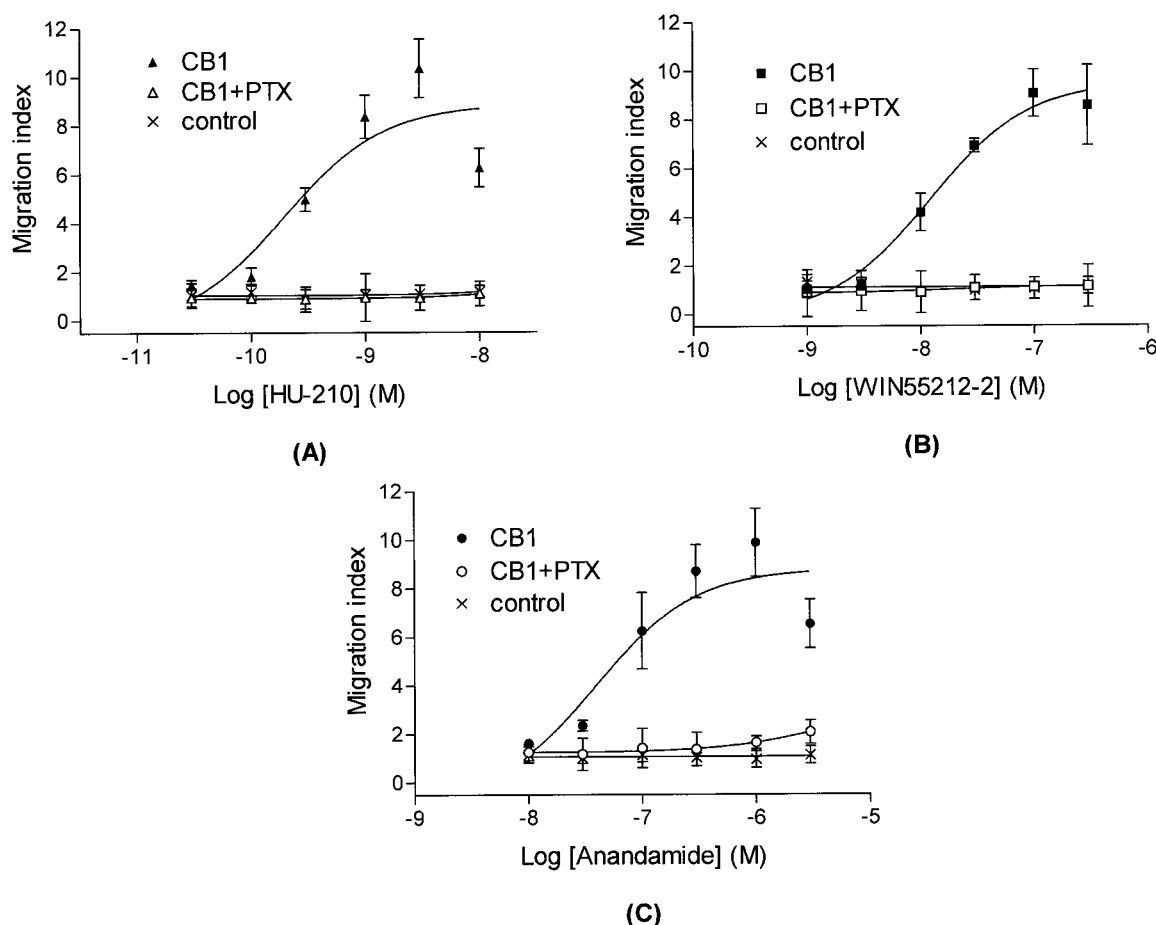


Fig. 1. Effects of HU-210 (A), WIN55212-2 (B), and anandamide (C) on migration of 293 cells stably transfected with human *CB1* gene. Cells were pretreated with 100 ng/ml pertussis toxin (PTX, open symbols) or vehicle (solid symbols) overnight before they were subject to cell migration assays. Data on vector-transfected 293 cell controls (x) were also shown. Results are expressed as migration index as described in *Experimental Procedures*. Data represent mean $\pm$ S.E. of three separate assays, each conducted in triplicate.

by two-way ANOVA). Pertussis toxin pretreatment at the same concentration was able to block cannabinoid-induced inhibition of forskolin-stimulated cAMP accumulation (data not shown).

Effects of a CB1 Antagonist on Cannabinoid Agonist-Induced Cell Migration. SR141716A, a selective CB1 antagonist (Rinaldi-Carmona et al., 1994), was used to test whether cannabinoid agonist-induced cell migration is mediated by specific CB1 receptors. In a concentration-dependent manner, SR141716A inhibited the cell migration induced by HU-210, WIN55212-2, and anandamide. The concentration-response curves were shifted parallel to the right by SR141716A ($P < .05$ by two-way ANOVA) (Fig. 2, A, B, and C). The pA_2 values for SR141716A against HU-210, WIN55212-2, and anandamide were 7.81 ± 0.20 , 8.09 ± 0.04 , and 7.92 ± 0.02 , respectively. SR141716A by itself had no significant effect on the cell migration (Fig. 2D).

Checkerboard Analysis of Anandamide-Induced Cell Migration. Checkerboard analysis was performed to examine the degree to which the migratory responses of cells are due to chemotactic or chemokinetic effects (Wilkinson, 1998; Zigmond and Hirsch, 1973). The results of checkerboard analysis are shown in Table 1. The underlined values along the diagonal reflect migratory responses to uniform concentrations of anandamide on both sides of the chamber (chemokinesis). The values below the diagonal reflect responses to a

positive gradient (chemotaxis), whereas the values above the diagonal represent responses to a negative gradient (chemokinesis). When equal concentrations of anandamide were present in the upper and lower chambers, the migration indexes were increased with increasing concentrations of anandamide ($P < .05$ by ANOVA). The migration indexes were increased with an increase in positive gradient of anandamide ($P < .05$ by ANOVA). These data demonstrate that anandamide induced both chemokinesis and chemotaxis, with chemotaxis stimulated to a greater extent (maximum of $7.79/1.12 = 7$ -fold increase over the background compared with a $5.54/1.12 = 5$ -fold increase for chemokinesis).

Effects of a Cell-Permeable Analog of cAMP on Anandamide-Induced Cell Migration. To study whether the anandamide-induced cell migration is a result of inhibition of adenylate cyclase, thereby a decrease in intracellular cAMP levels, the cells were pretreated with 8-Br-cAMP for 30 min before being subject to migration assays. As shown in Fig. 3A, this pretreatment did not block the migratory responses induced by anandamide. At 1 and 3 mM, 8-Br-cAMP might have enhanced cell migration. However, analysis by ANOVA demonstrated no significant differences ($P > .05$) between the data points in Fig. 3A. In the absence of cannabinoids, 8-Br-cAMP by itself had no significant effect ($P > .05$ by ANOVA) on the cell migration (Fig. 3B). In our control ex-

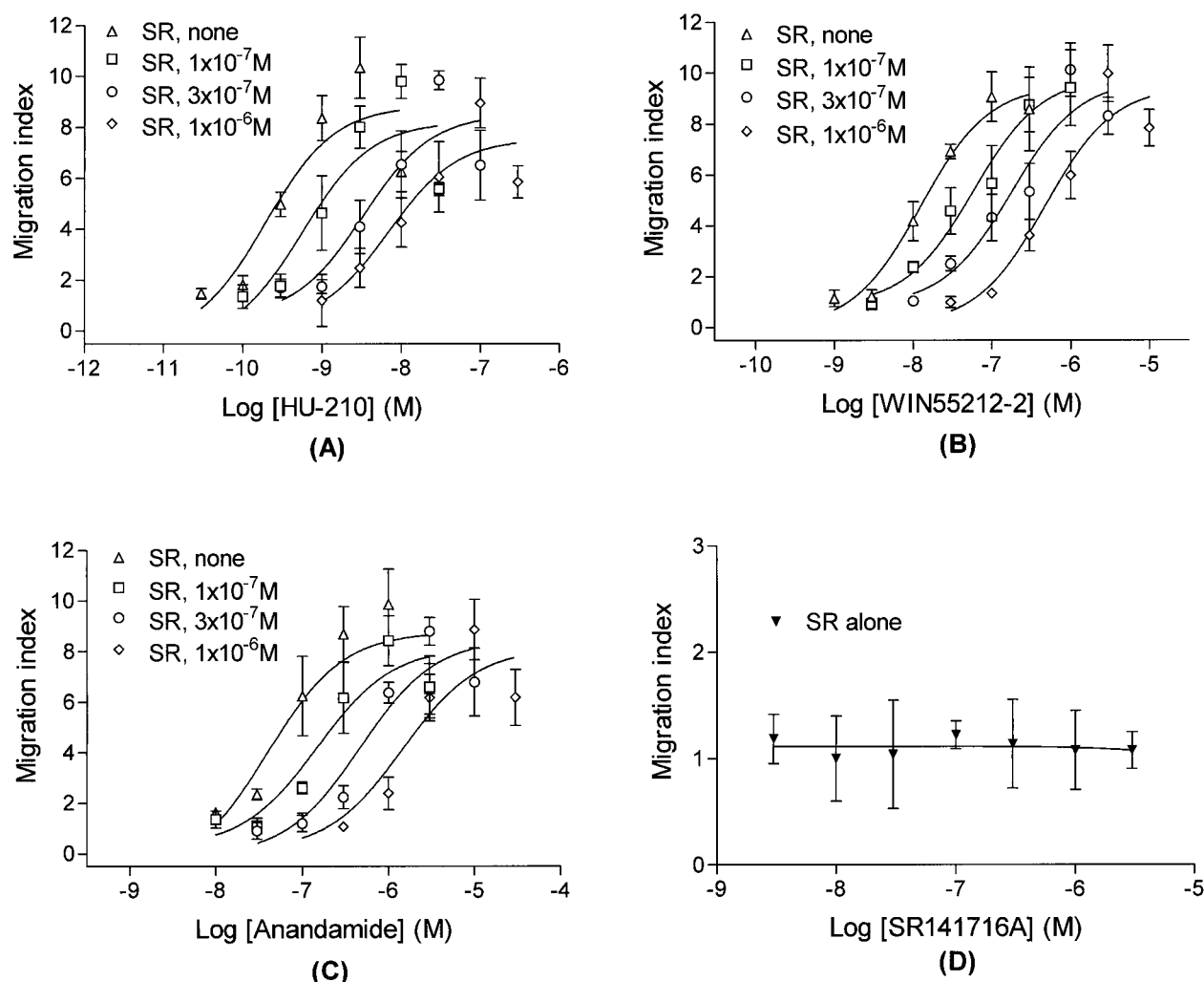


Fig. 2. Effects of SR141716A on cell migration induced by HU-210 (A), WIN55212-2 (B), and anandamide (C). Cells were pretreated with various concentrations of SR141716A for 30 min before they were subject to cell migration assays. Effects of SR141716A alone on cell migration are shown in (D). Results are expressed as migration index as described in *Experimental Procedures*. Data represent mean $\pm$ S.E. of three separate assays, each conducted in triplicate.

TABLE 1

Checkerboard analysis of anandamide-induced migration of 293 cells stably transfected with human *CB1* gene

Results are expressed as migration index as described in *Experimental Procedures*. Data represent mean $\pm$ S.E. of three separate assays, each conducted in triplicate.

Anandamide in Lower Chamber (M)	Anandamide in Upper Chamber (M)			
	0	3×10^{-8}	1×10^{-7}	3×10^{-7}
0	1.12 ± 0.13	1.23 ± 0.11	1.33 ± 0.11	1.13 ± 0.15
3×10^{-8}	2.33 ± 0.34	1.99 ± 0.26	2.27 ± 0.36	2.16 ± 0.47
1×10^{-7}	4.69 ± 0.65	4.05 ± 0.52	3.82 ± 0.53	3.85 ± 0.66
3×10^{-7}	7.79 ± 1.69	5.42 ± 1.07	5.51 ± 0.99	5.54 ± 1.04

periments, 8-Br-cAMP was active in that it enhanced the activation of MAPK by cannabinoids as previously demonstrated (Bouaboula et al., 1995) (data not shown).

Effects of an Inhibitor of MAPK Activation on Anandamide-Induced Cell Migration. MAPK, i.e., extracellular signal-regulated kinase (ERK)-1 and ERK-2, are a group of serine/threonine-specific protein kinases that are activated by various cell surface receptors, including receptor tyrosine kinases, receptors coupled to cytoplasmic tyrosine kinases, and G protein-coupled receptors (Hill and Treisman, 1995; Seger and Krebs, 1995). PD98059 is an inhibitor of MEK (MAPK or ERK kinase), a

dual specific kinase that activates both ERK-1 and ERK-2 by phosphorylation at specific threonine and tyrosine residues (Alessi et al., 1995). PD98059 was used to study the role of MAPK activation in anandamide-induced cell migration. In a concentration-dependent manner, PD98059 inhibited the anandamide-induced cell migration, with an IC_{50} value of $8.6 \pm 1.3 \mu\text{M}$ (Fig. 4A) ($P < .05$ by ANOVA). In the absence of cannabinoids, PD98059 by itself had no significant effect on the cell migration (Fig. 4B) ($P > .05$ by ANOVA). In our control experiments, PD98059 was able to inhibit cannabinoid-induced MAPK activity as expected (Alessi et al., 1995) (data not shown).

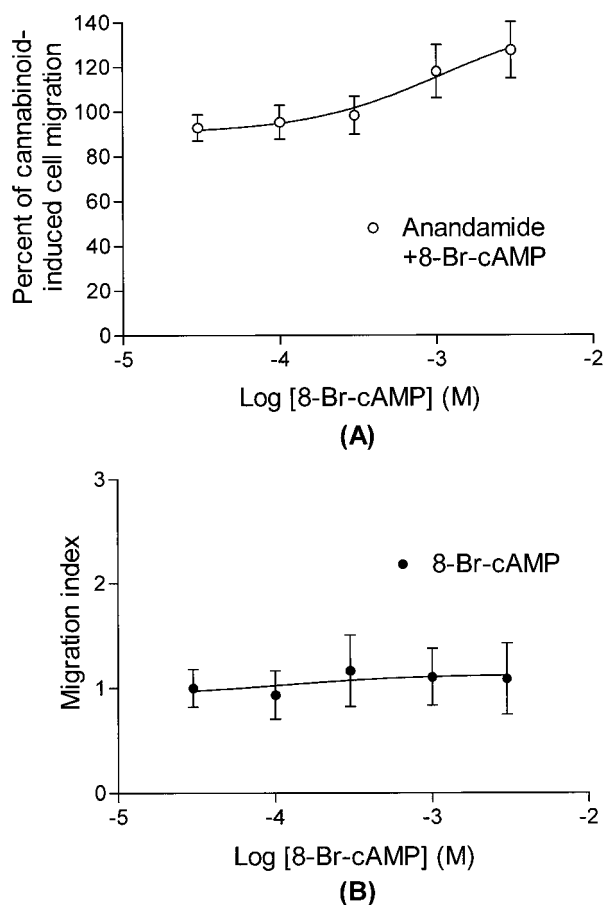


Fig. 3. Effects of 8-Br-cAMP on anandamide-induced cell migration (A). Cells were pretreated with various concentrations of 8-Br-cAMP or vehicle for 30 min before they were subject to the cell migration assays. Results are expressed as the percentage of anandamide-induced cell migration with 8-Br-cAMP pretreatment versus with vehicle pretreatment. Effects of 8-Br-cAMP alone on cell migration are shown in (B). Results are expressed as migration index as described in *Experimental Procedures*. Data represent the mean $\pm$ S.E. of three separate assays, each conducted in triplicate.

Discussion

In this study, HU-210, WIN55212-2, and anandamide, three cannabinoid agonists with distinct chemical structures, were found to cause the migration of 293 cells stably transfected with human *CB1* gene. In contrast, these cannabinoid agonists did not cause the migration of 293 cells stably transfected with an empty expression vector. The cannabinoid-induced cell migrations were concentration-dependent, with a rank order of potency of HU-210 > WIN55212-2 > anandamide. The concentration-response curves of cannabinoid agonists were shifted parallel to the right by SR141716A, a selective antagonist for *CB1*. These data indicate that the cannabinoid agonist-induced cell migratory responses are mediated by specific *CB1* receptors expressed in these cells.

The cannabinoid-induced migratory responses were impaired in this study by pretreatment of cells with 100 ng/ml pertussis toxin. These results demonstrate that pertussis toxin-sensitive G proteins are crucial for cannabinoid agonist-induced cell migration. These results are consistent with the previous reports that functional *CB1*s are coupled to G_i proteins (Howlett et al., 1986; Felder et al., 1992). These data also support the notion suggested by Arai et al. (1997) and

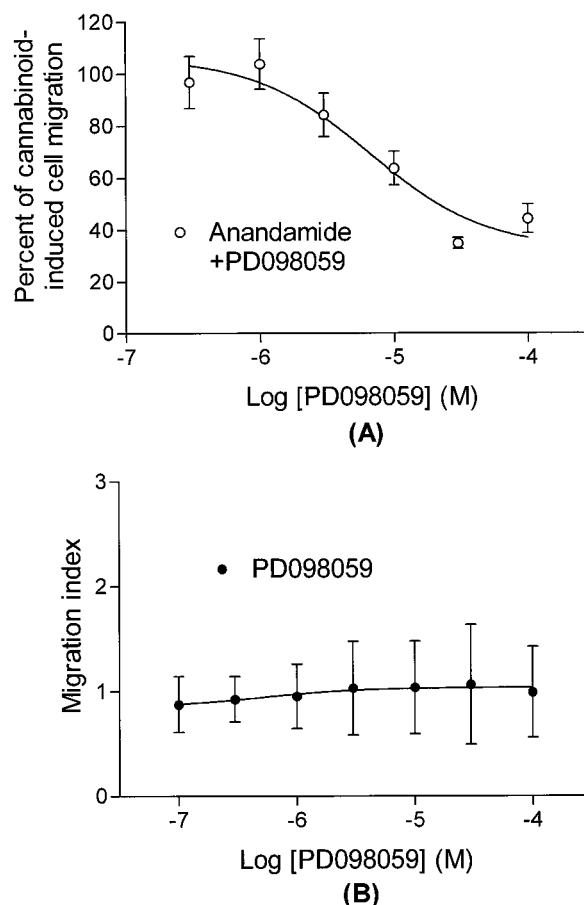


Fig. 4. Effects of PD098059 on anandamide-induced cell migration (A). Cells were pretreated with various concentrations of PD098059 or vehicle for 30 min before they were subject to the cell migration assays. Results are expressed as percentage of anandamide-induced cell migration with PD098059 pretreatment versus with vehicle pretreatment. Effects of PD098059 alone on cell migration are shown in (B). Results are expressed as migration index as described in *Experimental Procedures*. Data represent the mean $\pm$ S.E. of three separate assays, each conducted in triplicate.

Neptune and Bourne (1997) that the ability to mediate cell migration is shared by G_i -coupled receptors.

To identify the type of cell migration induced by anandamide, checkerboard analyses were carried out in this study. Our experiments demonstrated that anandamide is able to induce both chemotaxis and chemokinesis; however, chemotaxis was induced to a greater extent.

One of the known mechanisms of signal transduction for *CB1* is inhibition of adenylate cyclase (Howlett et al., 1986; Felder et al., 1992). However, in this study we found 8-Br-cAMP, a cell-permeable analog of cAMP, did not attenuate cannabinoid agonist-induced cell migration. These results indicate that cell migration is not mediated by a decrease of intracellular cAMP level induced by anandamide. This conclusion is consistent with the previous findings that cAMP is not a critical intracellular mediator of cell migration (Neptune and Bourne, 1997).

The well known roles of MAPK are their involvement in the signal transduction between the plasma membrane receptors and the nucleus (Hill and Treisman, 1995; Seger and Krebs, 1995). For G_i protein-coupled receptors, it has been shown that the activation of MAPK pathway is mediated by

$\beta\gamma$ subunit stimulation of Ras (Crespo et al., 1994; Koch et al., 1994). However, in certain cell types, G_i proteins use a novel $\beta\gamma$ - and Ras-independent pathway to activate MAPK (Hedin et al., 1999). At present, the roles of MAPK in cell migratory responses mediated by G protein-coupled receptors have not been established, and existing reports are controversial. For example, Kuroki and O'Flaherty (1997) reported that PD098059 blocks neutrophil chemotaxis induced by several chemoattractants. In contrast, Knall et al. (1997) reported that MAPK activation is not important in interleukin-8-induced chemotaxis. It has been established that activation of CB1 cannabinoid receptor increases the activity of MAPK (Bouaboula et al., 1995). In this study, PD098059 inhibited concentration dependently anandamide-induced cell migration. The effective concentrations of PD098059 in inhibiting cell migration are within the concentration range for this drug to inhibit MAPK activation (Alessi et al., 1995). Thus, these data suggest that activation of MAPK may play an important role in anandamide-induced cell migration. Currently, it is not clear how the MAPK activation is involved in anandamide-induced cell migration. Cytoskeletal regulation is a critical step in cell migration (Lauffenburger and Horwitz, 1996; Mitchison and Cramer, 1996). MAPK has been reported to regulate cytoskeleton and cell motility by phosphorylating and enhancing myosin light chain kinase activity (Klemke et al., 1997). Because it is known that anandamide can activate MAPK (Wartmann et al., 1995), one possible mechanism for anandamide-induced cell migration may be anandamide-induced activation of MAPK, and subsequent cytoskeletal regulation by MAPK. In this study, the inhibition by PD098058 was not complete. This suggests that in addition to MAPK, there might be other pathways that are involved in cannabinoid-induced cell migration.

After demonstrating the involvement of CB1 in cannabinoid-induced migration with three different agonists, we focused on anandamide because it is an endogenous cannabinoid agonist. We expect similar mechanisms for HU-210 and WIN55212-2 because it is well known that both of these ligands activate similar second messenger systems through CB1.

In summary, to our knowledge this is the first report that cannabinoid agonists can induce cell migration. These migratory responses are concentration-dependent, antagonized by SR141716A, a selective antagonist for the CB1, and blocked by pertussis toxin pretreatment. These results indicate that these migratory responses are mediated by G protein-coupled, CB1 cannabinoid receptors. Furthermore, anandamide-induced migratory responses are blocked by PD098059, an inhibitor of MAPK activation, but not by 8-Br-cAMP, a cell-permeable analog of cAMP. These data suggest that activation of MAPK, but not inhibition of adenylate cyclase, are crucial for the cell migration mediated by CB1. The detailed cellular and molecular mechanisms underlying the CB1-mediated cell migratory responses warrant additional investigation.

References

- Alessi DR, Cuenda A, Cohen P, Dudley DT and Saltiel AR (1995) PD098059 is a specific inhibitor of the activation of mitogen-activated protein kinase kinase in vitro and in vivo. *J Biol Chem* **270**:27489–27494.
- Arai H, Tsou CL and Charo IF (1997) Chemotaxis in a lymphocyte cell line transfected with C-C chemokine receptor 2B: Evidence that directed migration is mediated by $\beta\gamma$ dimers released by activation of G_i -coupled receptors. *Proc Natl Acad Sci USA* **94**:14495–14499.
- Bouaboula M, Poinot-Chazel C, Bourrie B, Canat X, Calandra B, Rinaldi-Carmona M, Le Fur G and Casellas P (1995) Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *Biochem J* **312**:637–641.
- Bouaboula M, Rinaldi M, Carayon P, Carillon C, Delpach B, Shire D, Le Fur G and Casellas P (1993) Cannabinoid-receptor expression in human leukocytes. *Eur J Biochem* **214**:173–180.
- Compton DR, Gold LH, Ward SJ, Balster RL and Martin BR (1992) Aminoalkylindole analogs: Cannabinomimetic activity of a class of compounds structurally distinct from delta-9-tetrahydrocannabinol. *J Pharmacol Exp Ther* **263**:1118–1126.
- Crespo P, Xu N, Simonds WF and Gutkind JS (1994) Ras-dependent activation of MAP kinase pathway mediated by G-protein $\beta\gamma$ subunits. *Nature (Lond)* **369**:418–420.
- Devane WA, Dysarz FA, Johnson MR, Melvin LS and Howlett AC (1988) Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol* **34**:605–613.
- Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A and Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science (Wash DC)* **258**:1946–1949.
- Falk W, Goodwin RH Jr and Leonard EJ (1980) A 48-well microchemotaxis assembly for rapid and accurate measurement of leukocyte migration. *J Immunol Methods* **33**:239–247.
- Felder CC, Veluz JS, Williams HL, Briley EM and Matsuda LA (1992) Cannabinoid agonists stimulate both receptor- and non-receptor-mediated signal transduction pathways in cells transfected with and expressing cannabinoid receptor clones. *Mol Pharmacol* **42**:838–845.
- Gerard CM, Mollereau C, Vassart G and Parmentier M (1991) Molecular cloning of a human brain cannabinoid receptor which is also expressed in testis. *Biochem J* **279**:129–134.
- Gerard G and Gerard NP (1996) The pro-inflammatory seven-transmembrane segment receptors of the leukocyte. *Curr Opin Immunol* **6**:140–145.
- Hedin KE, Bell MP, Huntoon CJ, Karnitz LM and McKean DJ (1999) G_i proteins use a novel $\beta\gamma$ - and Ras-independent pathway to activate extracellular signal-regulated kinase and mobilize AP-1 transcription factors in Jurkat T lymphocytes. *J Biol Chem* **274**:19992–20001.
- Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR and Rice KC (1991) Characterization and localization of cannabinoid receptors in rat brain: A quantitative in vitro autoradiographic study. *J Neurosci* **11**:563–583.
- Hill CS and Treisman R (1995) Transcriptional regulation by extracellular signals: Mechanisms and specificity. *Cell* **80**:199–211.
- Howlett AC, Quail JM and Khachatrian LL (1986) Involvement of G_i in the inhibition of adenylate cyclase by cannabinomimetic drugs. *Mol Pharmacol* **29**:307–313.
- Klemke RL, Cai S, Giannini AL, Gallagher PJ and de Lanerolle P (1997) Regulation of cell motility by mitogen-activated protein kinase. *J Cell Biol* **137**:481–492.
- Knall C, Worthen GS and Johnson GL (1997) Interleukin 8-stimulated phosphatidylinositol-3-kinase activity regulates the migration of human neutrophils independent of extracellular signal-regulated kinase and p38 mitogen-activated protein kinases. *Proc Natl Acad Sci USA* **94**:3052–3057.
- Koch WJ, Hawes BE, Allen LF and Lefkowitz RJ (1994) Direct evidence that G_i -coupled receptor stimulation of mitogen-activated protein kinase is mediated by $G_{\beta\gamma}$ activation of p21^{ras}. *Proc Natl Acad Sci USA* **91**:12706–12710.
- Kuroki M and O'Flaherty JT (1997) Differential effects of a mitogen-activated protein kinase inhibitor on human neutrophil responses to chemotactic factors. *Biochem Biophys Res Commun* **232**:474–477.
- Lauffenburger DA and Horwitz AF (1996) Cell migration: A physically integrated molecular process. *Cell* **84**:359–369.
- Mackie K and Hille B (1992) Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proc Natl Acad Sci USA* **89**:3825–3829.
- Matsuda LA, Bonner TI and Lolait SJ (1993) Localization of cannabinoid receptor mRNA in rat brain. *J Comp Neurol* **327**:535–550.
- Matsuda LA, Lolait SJ, Brownstein MJ, Young AC and Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature (Lond)* **346**:561–564.
- Mechoulam R, Feigenbaum JJ, Lander N, Segal M, Jarbe TU, Hiltunen AJ and Conroe P (1988) Enantiomeric cannabinoids: Stereospecificity of psychotropic activity. *Experientia* **44**:762–764.
- Mitchison TJ and Cramer LP (1996) Actin-based cell motility and cell locomotion. *Cell* **84**:371–379.
- Munro S, Thomas KL and Abu-Shaar M (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature (Lond)* **365**:61–65.
- Neptune ER and Bourne HR (1997) Receptors induce chemotaxis by releasing the $\beta\gamma$ subunit of G_i , not by activating G_q or G_{12} . *Proc Natl Acad Sci USA* **94**:14489–14494.
- Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Neliat G, Caput D, Ferrara P, Soubrie P, Breliere J-C and Le Fur G (1994) A potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett* **350**:240–244.
- Seger R and Krebs EG (1995) The MAPK signaling cascade. *FASEB J* **9**:726–735.
- Song ZH and Bonner TI (1996) A lysine residue of the cannabinoid receptor is critical for receptor recognition by several agonists but not WIN55212-2. *Mol Pharmacol* **49**:891–896.
- Wartmann M, Campbell D, Subramanian A, Burstein SH and Davis RJ (1995) The MAP kinase signal transduction pathway is activated by the endogenous cannabinoid anandamide. *FEBS Lett* **359**:133–136.
- Wilkinson PC (1998) Assays of leukocyte locomotion and chemotaxis. *J Immunol Methods* **216**:139–153.
- Zigmond SH and Hirsch JG (1973) Leukocyte locomotion and chemotaxis. *J Exp Med* **137**:387–410.

Send reprint requests to: Z. H. Song, Ph.D., Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY 40292. E-mail: zhsong@louisville.edu