The CB₁ Cannabinoid Receptor Is Coupled to the Activation of c-Jun N-Terminal Kinase

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ABSTRACT

Cannabinoids exert most of their effects through the CB₁ receptor. This G-protein-coupled receptor has been shown to functionally couple to inhibition of adenylyl cyclase, modulation of ion channels, and activation of extracellular signal-regulated kinase. Using Chinese hamster ovary cells stably transfected with the CB₁ receptor cDNA, we show here that ∆⁹-tetrahydrocannabinol (THC), the major active component of marijuana, induces the activation of c-Jun N-terminal kinase (JNK). Western blot analysis showed that both JNK-1 and JNK-2 were stimulated by THC. The effect of THC was also exerted by endogenous cannabinoids (anandamide and 2-arachidonoylglycerol) and synthetic cannabinoids (CP-55,940, HU-210, and methanandamide), and was prevented by the selective CB₁ antagonist SR141716. Pertussis toxin, wortmannin, and a Ras farnesyltransferase inhibitor peptide blocked, whereas mastoparan mimicked, the CB₁ receptor-evoked activation of JNK, supporting the involvement of a G₂₅-, protein, phosphoinositide 3'-kinase and Ras. THC-induced JNK stimulation was prevented by tyrphostin AG1296, pointing to the implication of platelet-derived growth factor receptor transactivation, and was independent of ceramide generation. Experiments performed with several types of neural cells that endogenously express the CB₁ receptor suggested that long-term JNK activation may be involved in THC-induced cell death. The CB₁ cannabinoid receptor was also shown to be coupled to the activation of p38 mitogen-activated protein kinase. Data indicate that activation of JNK and p38 mitogen-activated protein kinase may be responsible for some of the cellular responses elicited by the CB₁ cannabinoid receptor.

Cannabinoids, the active components of Cannabis sativa (marijuana) and their endogenous counterparts, exert most of their central and peripheral effects by binding to specific G-protein-coupled receptors (Howlett, 1995; Felder and Glass, 1998). To date, two different cannabinoid receptors have been characterized and cloned from mammalian tissues: CB₁ (Matsuda et al., 1990) and CB₂ (Munro et al., 1993). The CB₁ receptor is distributed mainly in the central nervous system but is also present in peripheral nerve terminals as well as in extraneuronal organs such as testis, uterus, spleen, and tonsils. By contrast, expression of the CB₁ receptor is almost exclusively restricted to cells and organs of the immune system (Matsuda et al., 1990; Munro et al., 1993; Felder and Glass, 1998). Several signaling pathways triggered by the activation of these receptors have already been described. For example, both the CB₁ and the CB₂ receptor signal inhibition of adenylyl cyclase and the CB₁ receptor is coupled to modulation of Ca²⁺ and K⁺ channels (Howlett, 1995; Felder and Glass, 1998). The recent discovery of a family of endogenous ligands of cannabinoid receptors (Devane et al., 1992; Di Marzo et al., 1994; Martin et al., 1999) and the potential therapeutic applications of cannabinoids (Voth and Schwartz, 1997) have focused a lot of attention on cannabinoids during the last years.

One of the most ubiquitous mechanisms of signal transduction in response to environmental stimuli is the activation of mitogen- and stress-activated protein kinase cascades (Minden and Karin, 1998; Garrington and Johnson, 1999). Members of the extracellular signal-regulated kinase (ERK) family are strongly activated by polypeptide growth factors whose receptors have tyrosine kinase activity and by tumor-promoting phorbol esters, whereas they are usually more weakly activated by stress stimuli and proinflammatory cytokines. In contrast, members of the c-Jun N-terminal kinase (JNK) and the p38 mitogen-activated protein kinase (MAPK) families are potently activated by stress signals but usually more modestly by polypeptide growth factors and phorbol esters. In particular, the widely distributed JNKs may be more strongly activated by polypeptide growth factors whose receptors have tyrosine kinase activity and by tumor-promoting phorbol esters, whereas they are usually more weakly activated by stress stimuli and proinflammatory cytokines. In contrast, members of the c-Jun N-terminal kinase (JNK) and the p38 mitogen-activated protein kinase (MAPK) families are potently activated by stress signals but usually more modestly by polypeptide growth factors and phorbol esters. In particular, the widely distributed JNKs may be strongly activated by polypeptide growth factors whose receptors have tyrosine kinase activity and by tumor-promoting phorbol esters, whereas they are usually more weakly activated by stress stimuli and proinflammatory cytokines. In contrast, members of the c-Jun N-terminal kinase (JNK) and the p38 mitogen-activated protein kinase (MAPK) families are more potently activated by stress signals but usually more modestly by polypeptide growth factors and phorbol esters.
JNK family members are activated after their phosphorylation on threonine and tyrosine residues by the dual-specificity upstream kinases MKK4 and MKK7. Once activated, JNK phosphorylates the transactivating domain of transcription factors, such as c-Jun, ATF2, and Elk-1, thereby increasing their stability and transcriptional activity. This in turn results in the control of the expression of genes that directly contribute to the mammalian stress response through changes in the cell cycle, DNA repair, or apoptosis (Ip and Davis, 1998; Minden and Karin, 1998; Garrington and Johnston, 1999).

In spite of the well-established role of JNK in the regulation of cell differentiation, proliferation, and death in the central nervous system, the possible coupling of the CB1 cannabinoid receptor to JNK has not been studied to date. However, several observations indicate that this may be a conceivable possibility: a) the CB1 cannabinoid receptor is coupled to ERK activation (Bouaboula et al., 1995a, b; Sánchez et al., 1998b), b) cannabinoids may induce anti proliferative effects through the CB1 receptor (De Petrocellis et al., 1998; Sánchez et al., 1998a; Chan et al., 1999; Galve-Roperh et al., 2000), and c) by releasing G-protein βγ-subunits, the CB1 cannabinoid receptor should be able to activate small G-proteins such as Ras and Rac, which lie upstream of the JNK cascade (Ip and Davis, 1998; Minden and Karin, 1998). The present work was therefore undertaken to test whether JNK is activated by the CB1 cannabinoid receptor.

Materials and Methods

Reagents. The following materials were kindly donated: Chinese hamster ovary (CHO) cells stably transfected with the rat CB1 cannabinoid receptor cDNA by Dr. T. I. Bonner (National Institutes of Health, Bethesda, MD) and Dr. Z. Vogel (The Weizmann Institute, Rehovot, Israel); SR 141716 by Sanofi Recherche (Montpellier, France); CP-55,940 by Dr. J. A. Ramos and Dr. J. J. Fernández-Ruiz (Complutense University, Madrid, Spain); HU-210 by Prof. R. France); and the anti-cannabinoids CP-55,940 and HU-210 were able to stimulate p38 MAPK as monitored by the incorporation of [γ-32P]ATP into specific substrates (c-Jun 1–169 and MAPK activated protein kinase-2 46–600) following dodecyl sulfate-polyacrylamide gel electrophoresis, autoradiography, and radioactive counting of the phosphorylated substrate bands according to manufacturer’s instructions (Upstate Biotechnology, Lake Placid, NY).

Western Blot Analysis of JNK Isoforms. Cells lysates were obtained as described above for determination of JNK activity. Samples were subjected to SDS-polyacrylamide gel electrophoresis in 10% gels, and proteins were transferred from SDS gels onto nitrocellulose membranes. The blots were then blocked with 5% fat-free dry milk in 50 mM Tris-HCl, pH 7.8, 100 mM NaCl, 0.1% Tween 20 (TBST). They were subsequently incubated for 1 h at 4°C with a monoclonal anti-phospho-Thr-183/Tyr-185-JNK antibody (1:1000 in TBST supplemented with 1% fat-free dry milk) that recognizes phosphorylated JNK-1 and JNK-2 isoforms (Santa Cruz Biotechnology, Santa Cruz, CA). After the blots were washed thoroughly, they were incubated with anti-mouse peroxidase-conjugated secondary antibody (1:10,000) for 1 h at 4°C and finally subjected to luminescence detection with an electrochemiluminescence detection kit. Loading controls were carried out with an anti-actin antibody.

Ceramide and Sphingomyelin Levels. Cells were transferred to chemically defined medium supplemented with 1 μCi of L-[3-14C]serine per well. After 48 h, reactions were started by the addition of the cannabinoids and were terminated after different times by aspiration of the medium and addition of 1 ml of methanol. Lipids were extracted and saponified, and ceramide and sphingomyelin were resolved by thin-layer chromatography in parallel with standards on silica-gel G60 plates with chloroform:methanol:water (100:42:6, v/v/v) as developing system until the front reached two-thirds of the plate. The solvent was then evaporated and plates were subsequently run with chloroform:methanol:acetic acid (94:1.5, v/v/v) until the front reached the top of the plate (Blázquez et al., 1999).

Statistical Analysis. Results shown represent the means ± S.D. of the number of experiments indicated in every case. Statistical analysis was performed by ANOVA. A post hoc analysis was made by the Student-Neuman-Keuls test.

Results

The CB1 Cannabinoid Receptor Is Coupled to JNK Activation. CHO cells stably transfected with the CB1 receptor cDNA constitute a well characterized model to study the signal transduction pathways modulated by this receptor. Here we used these cells to test the possible coupling of the CB1 receptor to JNK activation. CHO cells were treated for different times with THC, the major active component of marijuana. Cells were subsequently lysed, and JNK activity was determined. As shown in Fig. 1A, THC induced a time-dependent stimulation of JNK in CHO-CB1 cells. The effect of THC was transient, reaching a maximum at 5 to 10 min. Several experiments were subsequently performed to demonstrate the involvement of the CB1 receptor in the THC-induced stimulation of JNK in CHO-CB1 cells. a) Half-maximal stimulation of JNK by THC occurred at a concentration of ca. 5 nM (Fig. 1B), i.e., in the range of the Ki value of THC for the CB1 receptor (Howlett, 1995). b) The synthetic cannabinoids CP-55,940 and HU-210 were able to stimulate JNK to an extent similar to THC (Fig. 2). c) The stimulatory
The endogenous cannabinoids anandamide and 2-arachidonoylglycerol stimulated JNK to an extent higher than other cannabinoid agonists (Fig. 2). These endocannabinoids are known to be actively degraded by cellular fatty acid amidase hydrolysis to arachidonic acid and ethanolamine or glycerol, respectively (Martin et al., 1999). As shown in Fig. 2, in CHO-CB1 cells the effect of anandamide and 2-arachidonoylglycerol on JNK was not completely blocked by SR141716. In addition, in CHO-wt cells, anandamide and 2-arachidonoylglycerol induced a slight, although reproducible, activation of JNK. Methanandamide, a stable synthetic analog of anandamide (Martin et al., 1999), also stimulated JNK in CHO-CB1 cells. Moreover, arachidonic acid stimulated JNK in CHO-wt cells (151 ± 6% stimulation by 15 μM arachidonic acid, n = 3, P < .01 versus incubations with no additions; Fig. 2). These observations indicate that endocannabinoids stimulate JNK activity mostly by a CB1 receptor-dependent mechanism, with a minor contribution of a CB1 receptor-independent mechanism.

The two major JNK isoforms (i.e., JNK-1 and JNK-2) may become differentially activated by extracellular stimuli (cf. Ip and Davis, 1998; Minden and Karin, 1998). The contribution of these two isoforms to THC-induced JNK activation was assessed by Western blot with an antibody that recognizes phosphorylated (= activated) JNK-1 and JNK-2. As shown in Fig. 3, CHO cells expressed mostly the JNK-1 isoform. However, densitometric analysis of the lumograms showed that THC induced a similar increase in the phosphorylation of JNK-1 (33 ± 10% over incubations with no additions, n = 3) and JNK-2 (30 ± 6% over incubations with no additions, n = 3) in CHO-CB1 cells. This effect was not evident in CHO-wt cells and was prevented by SR141716 (Fig. 3).

The CB1 Cannabinoid Receptor Induces JNK Activation via a Gi/Go-Protein-, Phosphoinositide 3'-kinase (PI3K)-, and Ras-Dependent Pathway. The CB1 receptor is coupled to Gq-proteins (Howlett, 1995). To further investigate the signal transduction pathway responsible for JNK activation, the possible involvement of a Gq-protein was studied by examining the effect of pertussis toxin and mastoparan. As shown in Fig. 4, blockade of Gq/11-protein dissociation with pertussis toxin abrogated the THC-induced activation of JNK in CHO-CB1 cells. In addition, induction of Gq/11-protein dissociation with mastoparan induced a remarkable stimulation of JNK that was not additive to that exerted by THC.

It has been shown that wortmannin, a PI3K inhibitor, blocks the cannabinoid-induced stimulation of ERK (Bouaboula et al., 1997) and glucose metabolism (Sánchez et al., 1998b) in cells expressing the CB1 receptor. Class I PI3Ks, i.e., PI3K isoenzymes devoid of Src homology domains and activated by G-protein βγ-subunits, may mediate the activation of small G-proteins such as Ras and therefore stimulate the ERK cascade (Fruman et al., 1998). As shown in Fig. 4, the THC-induced activation of JNK in CHO-CB1 cells was fully prevented by wortmannin and the Ras farnesyltransferase inhibitor Cys-Val-2-naphthyl-3-alanyl-Met.

The CB1 Cannabinoid Receptor May Induce JNK Activation through Transactivation of Platelet-Derived Growth Factor (PDGF) Receptors. It is currently known that some G-protein-coupled receptors may mediate the activation of the Ras/ERK cascade via transactivation of receptors with tyrosine kinase activity, mostly epidermal growth factor (EGF) receptors (Hackel et al., 1999; Luttrell et al., 1999). For example, in cells expressing EGF receptors ERK activation evoked by lysophosphatidic acid is prevented by the tyrophostin AG1478, a selective inhibitor of EGF receptor tyrosine kinase. Alternatively, studies conducted with the tyrophostin AG1296, which selectively inhibits PDGF receptor tyrosine kinase, indicate that transactivation of this receptor may mediate lysophosphatidic acid-induced ERK activation in cells that do not express EGF receptors (Herrlich et al., 1998). CHO cells have been reported to express PDGF receptors (e.g., Duckworth and Cantley, 1997) but not EGF receptors (e.g., Morrison et al., 1993). Thus, as expected, in CHO-CB1 cells PDGF activated JNK, whereas EGF had no effect (Table 1). Moreover, the THC-induced stimulation of JNK was prevented by AG1296 but not by AG1478, pointing to the involvement of PDGF receptor transactivation.

The CB1 Cannabinoid Receptor Induces JNK Activation Independently of Ceramide. Ceramide plays an important role in the control of basic cell functions such as proliferation, differentiation, and death (Kolesnick and Krönke, 1998). Ceramide generation through sphingomyelin hy-
drolysis by acid sphingomyelinase is believed to mediate in
some instances the activation of the JNK cascade and in turn
may evoke changes in the cell proliferation/death decision
(Kolesnick and Krunke, 1998). However, exposure of CHO-
CB1 cells to THC (Fig. 5) or CP-55,940 (results not shown) did
not induce any significant change in the cellular levels of
sphingomyelin and ceramide. In addition, the acid sphingo-
myelinase inhibitor desipramine (50 μM, 90-min preincuba-
tion) did not prevent the THC-evoked activation of JNK
(results not shown).

**Cannabinoids Stimulate JNK in Cells Endogenously
Expressing the CB1 Receptor.** To test whether the canna-
binoid-induced stimulation of JNK observed in CHO cells
heterologously expressing the CB1 receptor could be extrap-
olated to cells naturally expressing this receptor, we exami-
ned the effect of cannabinoids on various neural cells that
express the CB1 receptor (Sánchez et al., 1998a; Galve-

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**Fig. 2.** The CB1 cannabinoid receptor is coupled to JNK activation. CHO
cells were incubated with or without 1 μM SR141716 (SR) for 30 min and
then with or without the cannabinoids [1 μM THC, 25 nM CP-55940 (CP),
25 nM HU-210, 15 μM anandamide (AEA), 15 μM 2-arachidonoylglycerol
(2-AG), or 15 μM methanandamide (M-AEA)] for an additional 5 min.
A, representative autoradiograms of c-Jun phosphorylation in CHO-CB1
and CHO-wt cells exposed to different additions. AA, arachidonic acid. B,
results from four different experiments in CHO-CB1 cells (closed bars)
and CHO-wt cells (open bars) are expressed as percentage of incubations
with no additions. Significantly different from incubations with no addi-
tions: *P < .01; **P < .05.

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**Fig. 3.** THC induces the phosphorylation of both JNK-1 and JNK-2.
CHO-CB1 cells and CHO-wt cells were incubated with or without 1 μM
SR141716 (SR) for 30 min and then with or without 1 μM THC for an
additional 5 min. Cell lysates were subsequently subjected to Western
blot with an anti-phospho-Thr-183/Tyr-185-JNK antibody that recog-
nizes phosphorylated JNK-1 and JNK-2. Loading controls were carried
out with an anti-actin antibody.

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**Fig. 4.** The CB1 cannabinoid receptor induces JNK activation via a
G/Gi/Go-protein, PI3K, and Ras-dependent pathway. CHO-CB1 cells were
incubated with or without 15 μM mastoparan (MAS, 20 min), 50 ng/ml
pertussis toxin (PTX, 14 h), 0.2 μM wortmannin (WM, 20 min), or 5 μM
Cys-Val-2-naphthyl-3-alanyl-Met (FTI, 14 h) and then with or without 1
μM THC for an additional 5 min. Results are expressed as percentage of
incubations with no additions and correspond to four different experi-
ments. Significantly different from incubations with no additions: *P < .01.
Inset, representative autoradiograms of c-Jun phosphorylation in
CHO-CB1 cells exposed to different additions.

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**TABLE 1**
The CB1 cannabinoid receptor may induce JNK activation via
transactivation of PDGF receptors

<table>
<thead>
<tr>
<th>Additions</th>
<th>JNK Activity</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100 ± 12</td>
<td></td>
</tr>
<tr>
<td>THC</td>
<td>168 ± 17*</td>
<td></td>
</tr>
<tr>
<td>PDGF</td>
<td>196 ± 19*</td>
<td></td>
</tr>
<tr>
<td>EGF</td>
<td>93 ± 11</td>
<td></td>
</tr>
<tr>
<td>AG1296</td>
<td>103 ± 4</td>
<td></td>
</tr>
<tr>
<td>AG1478</td>
<td>109 ± 11</td>
<td></td>
</tr>
<tr>
<td>THC + AG1296</td>
<td>112 ± 13</td>
<td></td>
</tr>
<tr>
<td>THC + AG1478</td>
<td>181 ± 26*</td>
<td></td>
</tr>
<tr>
<td>PDGF + AG1296</td>
<td>110 ± 19</td>
<td></td>
</tr>
</tbody>
</table>

Significantly different from incubations with no additions: * P < .01.
Roperh et al., 2000). As shown in Table 2, THC activated JNK acutely in two subclones of C6 glioma cells (namely, C6.9 and C6.4) that exhibit a distinct sensitivity to THC-induced apoptosis (Galve-Roperh et al., 2000). Short-term THC challenge induced a slight (although statistically significant) stimulation of JNK in U373 MG astrocytoma cells and primary astrocytes but not in primary neurons.

To test whether sustained JNK activation may be involved in the control of cell fate in our experimental system, cells were exposed to THC for 3 days, and JNK activity and cell viability were monitored. As shown in Table 2, viability was not significantly altered in cells in which JNK activity was either unaffected (primary neurons and astrocytes and CHO-CB1 cells) or modestly induced (glioma C6.4 and astrocytoma U373 MG) upon prolonged cannabinoid exposure. However, the remarkable induction of JNK activity observed in the glioma C6.9 was accompanied by a significant decrease in cell viability.

**The CB1 Cannabinoid Receptor Is Coupled to p38 MAPK Activation.** Compared to the readily activated JNKs, the p38 MAPK family members are usually activated by a more restricted array of stress stimuli. However, p38 MAPK plays an important role in cell response to osmotic and inflammatory stress (Herlaar and Brown, 1999). THC stimulated p38 MAPK in CHO-CB1 cells (Fig. 6) by 52% (n = 3, P < .01 versus incubations with no additions). This effect was prevented by SR141716 and was not evident in CHO-wt cells (Fig. 6), pointing to the involvement of the CB1 receptor. Cannabinoid-induced p38 MAPK activation, unlike cannabinoid-induced JNK activation, was not prevented by AG1296 (Fig. 6), indicating that PDGF receptor transactivation is not involved in the former effect.

**Table 2**
The CB1 cannabinoid receptor might control cell fate through sustained JNK activation.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>JNK Activity (%)</th>
<th>Cell Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10-min Incubation</td>
<td>72-h Incubation</td>
</tr>
<tr>
<td>Primary neurons</td>
<td>89 ± 6</td>
<td>79 ± 12</td>
</tr>
<tr>
<td>Primary astrocytes</td>
<td>126 ± 11**</td>
<td>114 ± 10</td>
</tr>
<tr>
<td>Glioma C6.9</td>
<td>167 ± 18*</td>
<td>359 ± 104*</td>
</tr>
<tr>
<td>Glioma C6.4</td>
<td>166 ± 20*</td>
<td>179 ± 10*</td>
</tr>
<tr>
<td>Astrocytoma U373 MG</td>
<td>119 ± 5**</td>
<td>161 ± 15*</td>
</tr>
<tr>
<td>CHO-CB1</td>
<td>176 ± 13*</td>
<td>83 ± 9</td>
</tr>
<tr>
<td>CHO-wt</td>
<td>94 ± 8</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D., not determined.

Significantly different from incubations with no additions: *P < .01; **P < .05.

**Discussion**

**Receptor Dependence of the Cannabinoid-Induced JNK Activation.** Data herein show that the CB1 cannabinoid receptor is coupled to JNK activation. This assumption is based mostly on the stimulation of JNK induced by synthetic and plant-derived cannabinoids in CHO-CB1 cells, but not in CHO-wt cells, as well as on the antagonism exerted by pertussis toxin and SR141716. We are aware that the latter compound has been shown to behave as an inverse agonist in a number of experimental models in vitro and in vivo (Bouaboula et al., 1997). However, we have been unable to demonstrate any effect of SR141716 on basal JNK activity in CHO-CB1 cells. The endocannabinoids anandamide and 2-arachidonoylglycerol seemed to exert part of their stimulatory action on JNK activity by a CB1 receptor-independent mechanism that may involve arachidonic acid release. This is in line with the arachidonic acid-induced stimulation of JNK reported by Shin et al. (1999).
Mechanism of JNK Activation. Many studies have demonstrated an involvement of the ERK and JNK cascades in the regulation of cell proliferation by G-protein-coupled receptors (Gutkind, 1998; Luttrell et al., 1999). Cannabinoid receptors are coupled to Gαi- and Gαs-proteins (Howlett, 1995). The βγ-subunits released from heterotrimeric Gαi- and Gαs-proteins are known to mediate the stimulation of small G-proteins such as Ras involved in the activation of the ERK and JNK cascades via class IA PI3Ks (Fruman et al., 1998; Minden and Karin, 1998; Luttrell et al., 1999). More recently, the α-subunits released from Gαi (Mochizuki et al., 1999) and Gαs-proteins (Jordan et al., 1999) have been shown to mediate ERK activation via a Ras- and PI3K-independent pathway. It is well established that Ras is the major small G-protein involved in the activation of the Raf-1/ERK cascade; however, the JNK cascade may also be activated by Rac and in some instances by Rho and Cdc42 (Minden and Karin, 1998; Yam-auchi et al., 1999). Although conflicting results exist concerning the relative importance of Ras and Rac in the activation of the JNK cascade, most likely both proteins are activatory components of the JNK cascade, Ras lying upstream of Rac (Scita et al., 1999). The CB1 cannabinoid receptor seems to be coupled to the activation of the ERK and JNK cascades through a common upstream mechanism involving Gαi/αs-protein βγ-subunits, class IA PI3K, and Ras. Current research is focused on the characterization of other elements linking the CB1 receptor to the JNK cascade, one of which could be focal adhesion kinase. This kinase, which has been shown to be phosphorylated by anandamide in primary neurons, is capable of activating small G-proteins such as Ras and may play an important role in the regulation of neuronal activity, plasticity, and survival (Girault et al., 1999).

Some G-protein-coupled receptors such as those for lysophosphatidic acid, thrombin, and angiotensin II may mediate the activation of the Ras/ERK cascade via transactivation of receptors with tyrosine kinase activity (Hackel et al., 1999; Luttrell et al., 1999). Our data indicate for the first time that the CB1 cannabinoid receptor may stimulate a protein kinase cascade such as the JNK cascade via transactivation of PDGF receptors. Interestingly, cannabinoid-induced stimulation of p38 MAPK was not prevented by AG1296, pointing to the existence of divergent pathways for JNK and p38 MAPK activation. Transactivation of EGF and PDGF receptors has been suggested to be the link between the release of Gαi/αs-protein βγ-subunits, leading to class IA PI3K/Src activation, and the stimulation of Ras via Shc/Grb-2/Sos (Hackel et al., 1999; Luttrell et al., 1999). Although cannabinoid receptors do not share a very high sequential homology with other receptors, they show a certain structural similarity with members of the EDG family of G-protein-coupled receptors (Yamaguchi et al., 1996). Ligands of these receptors include bioactive long-chain fatty acid derivatives such as lysophosphatidic acid and sphingosine 1-phosphate, whose structure resembles that of endocannabinoids. Lysophosphatidic acid and sphingosine 1-phosphate play an important role in the regulation of key cell functions such as proliferation, protection from apoptosis, differentiation, migration, and Ca2+ mobilization. Of interest, both lysophosphatidic acid receptors (Herrlich et al., 1998) and sphingosine 1-phosphate receptors (Pyne et al., 1999) are capable of stimulating ERK via transactivation of PDGF receptors.

Physiological Considerations. Although the actual biological functions of the endogenous cannabinoid system are as yet unknown, it is believed that endocannabinoids might play a role in brain development and function. Thus, the significance of the endogenous cannabinoid system is supported by the high levels of cannabinoid receptors found in brain; the specific mechanisms of endocannabinoid synthesis, uptake, and degradation in neural cells; and the neuromodulatory properties of endogenous cannabinoids (Felder and Glass, 1998; Martin et al., 1999). One of the most intriguing and unexplored actions of cannabinoids is their ability to control cell growth. Thus, cannabinoids have been shown to induce antiproliferative effects through the CB1 receptor in a number of cultured cell systems (De Petrocellis et al., 1998; Sánchez et al., 1998a; Chan et al., 1999; Galve-Roperh et al., 2000). Data in this report may help to explain the signal transduction mechanisms involved in cell growth control by cannabinoids. Thus, there might be a threshold above which cannabinoid-induced long-term JNK activation would lead to neural cell death. In the context of these findings, one could speculate that by modulating the balance among ERK, JNK, and p38 MAPK activities the CB1 cannabinoid receptor might regulate the fate of neural cells (regarding, e.g., proliferation, differentiation, and death) in response to environmental stimuli (cf. Datta and Greenberg, 1998; Derkinderen et al., 1999). Moreover, we have recently shown that cannabinoids are able to modulate through the CB1 receptor the activity of the PI3K/protein kinase B pathway, which serves as a pivotal antiapoptotic signal (Gómez del Pulgar et al., 2000). It is clear that further research is required to understand the physiological role of cannabinoids as modulators of cell fate.


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References


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