



## Cannabinoids and ceramide: Two lipids acting hand-by-hand

Guillermo Velasco, Ismael Galve-Roperh, Cristina Sánchez, Cristina Blázquez,  
Amador Haro, Manuel Guzmán\*

*Department of Biochemistry and Molecular Biology I, School of Biology, Complutense University, 28040 Madrid, Spain*

### Abstract

Cannabinoids, the active components of *Cannabis sativa* (marijuana) and their endogenous counterparts, exert their effects by binding to specific G-protein-coupled receptors that modulate adenylyl cyclase and ion channels. Recent research has shown that the CB<sub>1</sub> cannabinoid receptor is also coupled to the generation of the lipid second messenger ceramide via two different pathways: sphingomyelin hydrolysis and ceramide synthesis de novo. Sustained ceramide accumulation in tumor cells mediates cannabinoid-induced apoptosis, as evidenced by in vitro and in vivo studies. This effect seems to be due to the impact of ceramide on key cell signalling systems such as the extracellular signal-regulated kinase cascade and the Akt pathway. These findings provide a new conceptual view on how cannabinoids act, and raise interesting physiological and therapeutic questions.

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### Introduction

The hemp plant *Cannabis sativa* produces approximately 60 unique compounds known as cannabinoids, of which  $\Delta^9$ -tetrahydrocannabinol (THC) is the most studied owing to its high potency and abundance in cannabis (Gaoni and Mechoulam, 1964). THC exerts a wide variety of biological effects by mimicking endogenous substances – the endocannabinoids anandamide (Devane et al., 1992) and 2-arachidonoylglycerol (Mechoulam et al., 1995; Sugiura et al., 1995) – that bind to and activate specific cannabinoid receptors. So far, two cannabinoid-specific G<sub>i/o</sub>-protein-coupled receptors, CB<sub>1</sub> (Matsuda et al., 1990) and CB<sub>2</sub> (Munro et al., 1993), have been cloned and characterized from

\* Corresponding author. Tel.: +34 913944668, fax: +34 913944672.

E-mail address: [mgp@bbml.ucm.es](mailto:mgp@bbml.ucm.es) (M. Guzmán).

mammalian tissues. Most of the effects of cannabinoids rely on CB<sub>1</sub> receptor activation. This receptor is particularly abundant in discrete areas of the brain, but is also expressed in peripheral nerve terminals and various extra-neural sites such as testis, eye, vascular endothelium and spleen. In contrast, the CB<sub>2</sub> receptor is almost exclusively present in the immune system (Howlett et al., 2002).

Extensive molecular and pharmacological studies have demonstrated that cannabinoids inhibit adenylyl cyclase through CB<sub>1</sub> and CB<sub>2</sub> receptors. The CB<sub>1</sub> receptor also modulates ion channels, inducing, for example, inhibition of N- and P/Q-type voltage-sensitive Ca<sup>2+</sup> channels and activation of G protein inwardly rectifying K<sup>+</sup> channels (Howlett et al., 2002; Piomelli, 2003). Besides these well-established cannabinoid receptor-coupled events, cannabinoid receptors have also been shown to modulate several signalling pathways that are more directly involved in the control of cell proliferation and survival, including extracellular signal-regulated kinase (ERK) (Bouaboula et al., 1995), c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) (Liu et al., 2000; Rueda et al., 2000), phosphatidylinositol 3-kinase (PI3K)/Akt (Gómez del Pulgar et al., 2000), focal adhesion kinase (Derkinderen et al., 1996), and the ceramide pathway — the focus of this article (Guzmán et al., 2001).

Ceramide is a ubiquitous sphingolipid second messenger that plays an important role in the control of cell fate at different sites, including the central nervous system (Hannun and Obeid, 2002; Kolesnick, 2002). Thus, exposure of neural cells to physical, chemical, bacterial or viral stimuli may increase intracellular ceramide levels and therefore evoke changes in the cell survival/death decision (Goswami and Dawson, 2000). Changes in ceramide metabolism also exert important regulatory effects on neuronal growth and development (Buccoliero and Futerman, 2003). The aim of this article is to examine this aspect of cannabinoid-mediated signal transduction, and to discuss its potential physiological and therapeutic implications.

## **Cannabinoid-induced acute ceramide generation**

### *Mechanism*

Ceramide generation occurring within a time interval of minutes depends on the catalytic action of sphingomyelinases, which hydrolyse sphingomyelin to ceramide and phosphorylcholine (Kolesnick, 2002). CB<sub>1</sub> receptor activation induces sphingomyelin hydrolysis in both primary astrocytes (Sánchez et al., 1998b; Blázquez et al., 1999) and glioma cells (Sánchez et al., 1998a; Galve-Roperh et al., 2000), with a maximal effect at ~ 15 min. As expected, this stimulation is concomitant to an increase in ceramide levels (maximum twofold at 15 min). The functional coupling of receptors to sphingomyelinases may involve different adaptor proteins. One of these is the factor associated with neutral sphingomyelinase activation (FAN). FAN binds to a cytoplasmic nine-amino acid motif of the 55-kDa tumour necrosis factor (TNF) receptor, the so-called neutral sphingomyelinase-activating domain, thereby coupling the receptor to sphingomyelin breakdown (Adam-Klages et al., 1996; Segui et al., 2001). A role for FAN in CB<sub>1</sub> receptor-evoked sphingomyelin hydrolysis is supported by coimmunoprecipitation experiments evidencing the binding of FAN to the activated CB<sub>1</sub> receptor and by the resistance of cells expressing dominant-negative FAN to cannabinoid-induced sphingomyelin breakdown (Sánchez et al., 2001b). Of interest, both G-protein β subunits and FAN are members of the WD-repeat protein family (Adam-Klages et al., 1996). The WD repeat, which comprises a 44- to 60-

residue sequence that typically contains the WD dipeptide at the C-terminus, facilitates defined protein–protein interactions. In fact, many known WD-repeat proteins are regulatory or adaptor proteins involved in signal transduction (Smith et al., 1999).

The potential association of FAN to the CB<sub>1</sub> receptor is strengthened by the homology between a sequence in the 55-kDa TNF receptor FAN-binding domain (DSAHK) and a sequence in the cytoplasmic region of the CB<sub>1</sub> receptor (DCLHK). This homology is higher than that shared by the 55-kDa TNF receptor FAN-binding domain (EDSAH) and the sequence proposed to bind FAN in the immunoregulatory transmembrane protein CD40 (QETLH) (Segui et al., 1999). Another G-protein-coupled-receptor – the receptor for the chemokine growth-related gene product  $\beta$  – has been reported to evoke sphingomyelin hydrolysis through sphingomyelinase activation (Limatola et al., 1999). In summary, cannabinoid-induced acute ceramide generation might rely on sphingomyelin breakdown via FAN (Fig. 1).

### Function

The early peak of ceramide induced by cannabinoids has been linked to the regulation of metabolic functions (Fig. 1). Thus, cannabinoids stimulate the utilization of glucose, the main source of brain energy metabolism, and the production of ketone bodies, an alternative source of energy when glucose deprivation ensues, in primary astrocytes (Guzmán and Sánchez, 1999). Both effects are prevented by CB<sub>1</sub> receptor antagonist and are preceded by a rapid and transient increase in the levels of ceramide. Ceramide in turn seems to mediate the stimulation of glucose consumption via the ERK cascade (Sánchez et al., 1998b), and the stimulation of ketogenesis via the outer-mitochondrial-membrane carnitine palmitoyltransferase, the pace-setting enzyme for fatty acid oxidation (Blázquez et al., 1999;

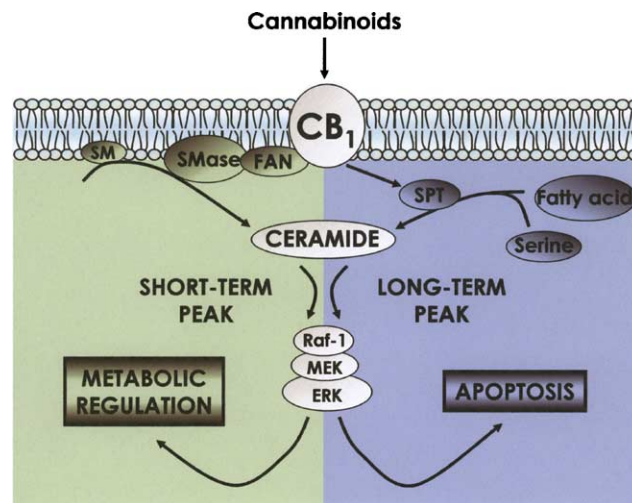


Fig. 1. The CB<sub>1</sub> cannabinoid receptor is coupled to ceramide generation. Activation of the CB<sub>1</sub> receptor can produce two peaks of ceramide. Short-term ceramide generation involves sphingomyelin (SM) hydrolysis via sphingomyelinase (SMase) activation possibly through the adaptor protein FAN. Long-term ceramide generation may occur via serine palmitoyltransferase (SPT) induction and enhanced ceramide synthesis de novo. The two pools of ceramide elicit biological responses (e.g. metabolic regulation and apoptosis) through regulation of various targets, for example the Raf-1/MEK/ERK cascade.

Guzmán and Blázquez, 2001). One of the most important functions of astrocytes is the regulation of brain energy metabolism by providing neurons with anaplerotic metabolites and substrates for generation of energy. It is therefore tempting to speculate that the endogenous cannabinoid system might regulate via ceramide the amount and type of nutrients supplied by astrocytes to neurons as source of carbon for neuronal biosynthetic processes (e.g. myelination) and oxidative metabolism (e.g. synaptic activity).

## **Cannabinoid-induced sustained ceramide generation**

### *Mechanism*

It is usually considered that ceramide generation through sphingomyelin hydrolysis is the norm in ceramide signalling pathways. However, long-term ceramide accumulation through enhanced synthesis de novo or impaired clearance and/or metabolism has been gaining appreciation as alternative means of generating a signalling pool of ceramide (Hannun and Obeid, 2002; Perry, 2002). In this context, cannabinoid receptor activation also evokes sustained ceramide accumulation. In glioma cells, THC and other cannabinoids induce a second peak of ceramide starting at days 2–3 of treatment and reaching a maximal fourfold increase at day 5 (Galve-Roperh et al., 2000). Serine palmitoyltransferase, the pace-setting enzyme for ceramide synthesis de novo, seems to be involved in this effect as indicated by measurements of enzyme activity and by the use of selective enzyme inhibitors (Fig. 1) (Gómez del Pulgar et al., 2002a). Cannabinoids have also been shown to induce ceramide synthesis de novo in other cell systems (Mimeault et al., 2003; Ramer et al., 2003). In addition, other studies support the view that, like stress stimuli, G-protein-coupled receptors may generate a functionally active pool of ceramide via enhanced synthesis de novo (Lehtonen et al., 1999).

### *Function*

An exciting aspect of current cannabinoid research is the possibility that these compounds participate in the control of the cell survival/death decision (Guzmán, 2003). The important role of ceramide in the induction of apoptosis prompted us to explore such a hypothesis in glial cells. Experiments carried out using different glioma cell subclones showed that sustained but not acute ceramide generation is responsible for cannabinoid-induced apoptosis (Fig. 1) (Galve-Roperh et al., 2000; Gómez del Pulgar et al., 2002b). Likewise, primary astrocytes, in which CB<sub>1</sub> receptor activation evokes acute but not sustained ceramide generation, are resistant to the apoptotic effect of cannabinoids (Sánchez et al., 1998a), but when ceramide synthesis de novo is induced selectively by palmitate loading they undergo apoptosis (Blázquez et al., 2000). Moreover, in contrast with their proapoptotic effect on glioma cells, cannabinoids protect normal glial cells of astroglial (Gómez del Pulgar et al., 2002a; Carracedo et al., 2004) and oligodendroglial (Molina-Holgado et al., 2002) lineages from apoptosis as induced by various toxic stimuli, including ceramide exposure. This protective effect is mediated by the CB<sub>1</sub> receptor and the PI3K/Akt survival pathway (Fig. 2) (Gómez del Pulgar et al., 2002a; Molina-Holgado et al., 2002).

The essential role of the Raf-1/MEK/ERK cascade as the target for de novo-synthesized ceramide in the induction of apoptosis has been evidenced in both glioma cells (Galve-Roperh et al., 2000) and

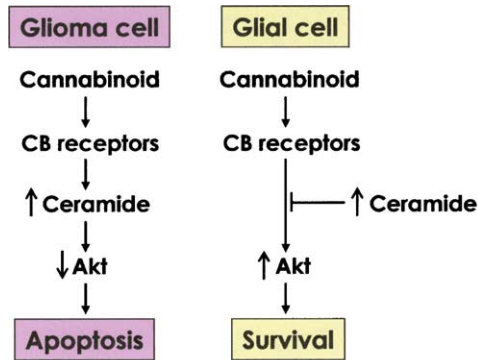


Fig. 2. Dual effect of cannabinoids on the viability of transformed versus non-transformed glial cells. In glioma cells cannabinoids activate CB receptors, inducing sustained ceramide accumulation and inhibition of the pro-survival kinase Akt, leading in turn to apoptosis. In normal glial cells cannabinoids activate CB receptors, preventing ceramide-induced Akt inhibition and leading in turn to cell survival.

primary astrocytes (Blázquez et al., 2000). It is generally accepted that ERK activation leads to cell proliferation. However, the relation between ERK activation and cell fate is complex and depends on many factors, one of which is the duration of the stimulus as prolonged cannabinoid-induced ERK activation may mediate cell cycle arrest (Melck et al., 1999) and cell death (Galve-Roperh et al., 2000). Sustained Akt inhibition (Gómez del Pulgar et al., 2002a) and JNK and p38 MAPK activation (Galve-Roperh et al., 2000; Rueda et al., 2000) may also contribute to glioma cell death. Nevertheless, further investigation is necessary to clarify the specific ceramide-regulated targets involved in cannabinoid-induced apoptosis.

It is worth noting that plant-derived and synthetic cannabinoids not only induce apoptosis of glioma cells in culture but also inhibit the growth of gliomas (Galve-Roperh et al., 2000) and other types of tumors (Bifulco and Di Marzo, 2002; Guzmán, 2003; Kogan et al., 2004) in laboratory animals without significant side-effects. Besides the aforementioned proapoptotic effect, immunohistochemical and functional analyses in mouse models of gliomas (Blázquez et al., 2003) and skin carcinomas (Casanova et al., 2003) have shown that cannabinoid administration turns the vascular hyperplasia characteristic of actively growing tumours to a pattern of blood vessels characterized by small, differentiated and impermeable capillaries. This is associated with a reduced expression of vascular endothelial growth factor (VEGF) and other proangiogenic cytokines, as well as of VEGF receptors (Blázquez et al., 2003, 2004; Casanova et al., 2003; Portella et al., 2003). Pharmacological inhibition of ceramide synthesis de novo abrogates the antitumoural effect of cannabinoids in vivo as well as the cannabinoid-induced inhibition of VEGF production by glioma cells in vitro and by gliomas in vivo (C. Blázquez et al., 2004), indicating that ceramide plays a general role in cannabinoid antitumoural action. Of interest, ceramide content has been inversely related with malignant progression and poor prognosis of human astrocytomas. Thus, low grade astrocytomas have higher ceramide content than high grade astrocytomas (Riboni et al., 2002). Moreover, it has been shown that low grade astrocytomas have lower CB<sub>2</sub> receptor expression than high grade astrocytomas (Sánchez et al., 2001a), suggesting that this particular cannabinoid receptor subtype may be a marker for brain tumour malignancy. In the context of the “sphingolipid rheostat” theory (Hannun and Obeid, 2002; Kolesnick, 2002), the mitogenic sphingolipid sphingosine 1-phosphate would shift the balance towards angiogenesis and

tumorigenesis (Spiegel and Milstein, 2003), whereas the antiproliferative sphingolipid ceramide would blunt angiogenesis and tumorigenesis.

### Potential implications

The CB<sub>1</sub> cannabinoid receptor, besides its well known coupling to modulation of adenylyl cyclase and ion channels through G<sub>i/o</sub> proteins, is coupled to the generation of the lipid second messenger ceramide. Studies in glioma cells show that upon CB<sub>1</sub> receptor activation two peaks of ceramide generation are observed which have different kinetics (minute- versus day-range), magnitude (two- versus four-fold), mechanistic origin (sphingomyelin hydrolysis versus ceramide synthesis de novo) and function (metabolic regulation versus induction of apoptosis) (Fig. 1). These observations open a new conceptual view on how cannabinoids act, and anticipate important physiological implications in view of the emerging role of ceramide in the control of cell fate and the increasing array of cell functions modulated by cannabinoids. For example, cannabinoids – like other ceramide-generating agents (Radin, 2003) – might be considered as potential therapeutic drugs for the management of malignant tumors. In addition, the endogenous cannabinoid system has been suggested to play a specific role in brain development (Fernández Ruiz et al., 2000). In this context, endocannabinoids modulate the differentiation program of neural progenitors by decreasing the fraction of newly-born cells that differentiate into neurons (Rueda et al., 2002). Other experimental evidences support that endocannabinoids participate in the regulation of neuritogenesis (Zhou and Song, 2001; Ishii and Chun, 2002), axonal growth (Williams et al., 2003) and synaptogenesis (Kim and Thayer, 2001). All these observations suggest that endocannabinoids constitute a new family of lipid signalling cues responsible for the regulation of neural cell development and survival, and provide a conceptual and mechanistic basis for the effects of marijuana-derived cannabinoids. Further research is necessary, however, to determine the real impact of cannabinoids on brain development and the possible involvement of ceramide in these events.

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