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Letter

Phytocannabinoids, Δ^9-tetrahydrocannabinol and cannabidiol, as human calpain-1 (CAPN1) activators

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ABSTRACT — The effects of Δ^9-tetrahydricannabinol (Δ^9-THC), cannabidiol (CBD), cannabidiolic acid (CBDA), and 2-methyl-2'-fluoro-anandamide (MF-AEA, a stable analog of anandamide) on the enzymatic activity of purified human calpain-1 (CAPN1) were investigated in the present study. Although leupeptin, a calpain inhibitor, reduced calpain-1 activity in a dose-dependent manner (1, 5, and 25 μM), among the four cannabinoids tested, Δ^9-THC and CBD exerted stimulatory effects on calpain-1 enzymatic activity in the same concentration range as leupeptin. CBDA and MF-AEA did not modulate calpain-1 activity, indicating that the phenol structure in Δ^9-THC/CBD is a key point, and the carboxylic acid moiety appears to be negatively involved. This is the first study to show that the phytocannabinoids, Δ^9-THC and CBD have the ability to activate the enzymatic activity of human calpain-1.

Key words: Δ^9-Tetrahydricannabinol, Cannabidiol, Calpain-1, CAPN1, 2-Methyl-2'-fluoro-anandamide

INTRODUCTION

The plant *Cannabis sativa* is one of the oldest known plants cultivated by human community and produces pharmacologically active compounds that have not been detected in any other plants (Yamauchi *et al*., 1968; Turner *et al*., 1980; Taura *et al*., 2007; Izzo *et al*., 2009). Among them, the most important cannabinoids are Δ^9-tetrahydricannabinol (Δ^9-THC) and cannabidiol (CBD), which are present as major cannabinoids in the *Cannabis* plant (Yamauchi *et al*., 1968; Turner *et al*., 1980; Taura *et al*., 2007; Izzo *et al*., 2009). Many preclinical studies have suggested the usefulness of Δ^9-THC and CBD for abrogating the progression of cancer (possibly as anti-cancer agents) *in vitro* and *in vivo* (Izzo *et al*., 2009; Caffarel *et al*., 2012). Dronabinol (a synthesized Δ^9-THC) is now used to buffer side effects, such as nausea and vomiting, associated with cancer therapy in some countries (Caffarel *et al*., 2012). Although cannabinoids including dronabinol are used for the above-mentioned palliative purposes in cancer therapy, those are limited to use as "anti-cancer drugs". Furthermore, nabiximols (Sativex, an oromucosal spray containing an almost equal 1:1 ratio of Δ^9-THC/CBD) have been approved as a botanical drug and are used to suppress neuropathic pain in clinical settings (Izzo *et al*., 2009; Caffarel *et al*., 2012).

Calpains are intracellular calcium-dependent cysteine proteases, and, among calpain family members, calpain-1 (CAPN1) is known as a ubiquitous calpain (Green and Kroemer, 1998; Ono and Sorimachi, 2012). Since calpains have been implicated in various pathophysiological settings, chemicals that target their activities may be candidates for the production of drugs to treat diseases. Calpains may be activated via at least two mechanisms: (i) chemicals initially stimulate cellular signaling, which leads to the activation of calpain enzymes (*i.e.*, possibly indirect activation), and (ii) the chemically-based direct activation of calpains. When compared with the proposed mechanism (i), chemicals involved in mechanism (ii) are very limited; *e.g.*, ONO-3403, a synthetic serine protease inhibitor, has been shown to stimulate the activity of purified calpain-1 at a concentration higher than 180 μM (Hiwasa, 1996). A previous study indicated that the botanical extracts nabiximols (Δ^9-THC/CBD)

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delayed disease progression in rat models of Huntington's disease through, at least in part, the up-regulation of calpain expression (Sagredo *et al*., 2011). Furthermore, in the field of cancer prevention, the expression of calpain-1 has been positively associated with relapse-free survival in some breast cancer patients treated with trastuzumab and chemotherapy (Storr *et al*., 2011), and anandamide (AEA), an endogenous cannabinoid, also has the potential to activate calpain through cannabinoid receptor-independent signaling in rat C6 glioma cells (Jacobsson *et al*., 2001). However, there is currently no experimental evidence to show whether phyto/endocannabinoids (Δ^9-THC, CBD, and AEA) directly affect the enzymatic activity of calpain-1. In the present study, we utilized human calpain-1 as an enzyme source, and demonstrated that the phytocannabinoids, Δ^9-THC and CBD, stimulated the enzymatic activity of human calpain-1 to a similar extent, even at a concentration of 1 μM.

MATERIALS AND METHODS

Materials

Δ^9-THC, CBD, and CBDA were isolated from cannabis leaves by established methods (the purities of Δ^9-THC, CBD, and CBDA were > 98%) (Takeda *et al*., 2008), and commercially available CBD and CBDA samples (purity: > 98% and 96.5%, respectively) were also used (form Tocris Bioscience, Ellisville, MO, USA and Sigma-Aldrich, St. Louis, MO, USA, respectively). No significant differences were observed in the biological activities of these CBD/CBDA samples. 2-Methyl-2'-fluoro-AEA (MF-AEA, a stable analog of AEA) was purchased from Cayman Chemical (Ann Arbor, MI, USA). All other reagents were of analytical grade, commercially available, and used without further purification.

Calpain activity assay

Calpain activity in the presence or absence of cannabinoids or leupeptin (a calpain inhibitor) in combination with 2 mM calcium was assessed using Calpain-Glo™ Protease Assay reagents (Promega, Madison, WI, USA), according to the manufacturer's instructions in the GloMax-Multi Detection System (Promega). In this assay, we used human calpain-1 purified from erythrocytes (EMD Biosciences, La Jolla, CA, USA).

Data analysis

Differences were considered significant when the *P* value was calculated to be less than 0.05. A data analysis of differences among multiple groups was performed using Dunnett's test. These calculations were performed using Statview 5.0J software (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

In the present study, we utilized four cannabinoids, Δ^9-THC, CBD, CBDA (the acid form of CBD), and MF-AEA (Fig. 1A), and investigated their effects on the enzymatic activity of purified human calpain-1. Leupeptin, a calpain inhibitor, abrogated calcium-stimulated calpain-1 activity in a concentration-dependent manner (1, 5, and 25 μM), indicating that calpain-1 was sufficiently activated under these conditions (Fig. 1B). Based on this result, cannabinoids were applied to the calpain-1 enzyme sys-

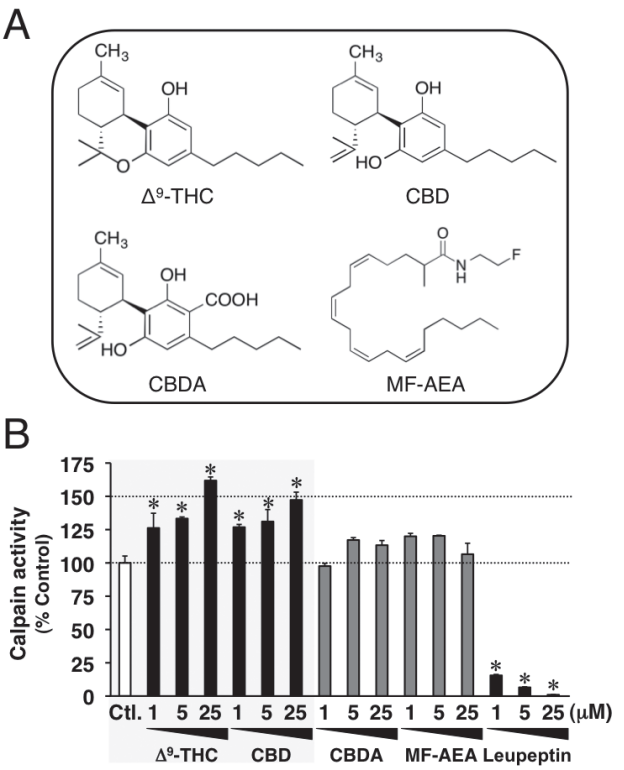

Fig. 1. Effects of phyto/endocannabinoids (Δ^9-THC, CBD, and CBDA/MF-AEA) on calpain-1 activity. (A) Structures of the cannabinoids tested. (B) Δ^9-THC and CBD were potent stimulators of calpain-1. Reactions mediated by human calpain-1 were performed in the presence or absence (vehicle control; Ctl.) of the four cannabinoids (1, 5, and 25 μM). Assays for leupeptin were conducted to assess the sufficient activation of calpain-1. Details on assay conditions are described under *Materials and Methods*. Data are expressed as a percentage of the vehicle-treated group, as the mean ± S.E. (n = 3) *Significantly different from ($P < 0.05$) from the control.

tem at concentrations of 1, 5, and 25 μM. As shown in Fig. 1B, calpain-1 was significantly activated by Δ^9-THC and CBD in the same concentration range as leupeptin, but not by CBDA or MF-AEA.

The inability of CBDA/MF-AEA to activate calpain-1 suggests that the phenolic structure in Δ^9-THC/CBD is important, and the carboxylic acid moiety appears to be negatively involved (see Fig. 1A). The mechanisms by which Δ^9-THC and CBD stimulate the activity of calpain-1 currently remain unclear. In structural comparisons with ONO-3403, a direct activator of calpain-1 (Hiwasa, 1996), no similarities were observed between Δ^9-THC/CBD and ONO-3403. Since the whole crystal structure of human calpain-1 together with calcium has not yet been elucidated, we cannot provide rational evidence for the activation mechanism(s) used by these two cannabinoids. The Δ^9-THC concentrations of 1-25 μM used in the present study may reflect therapeutically relevant conditions after its administration (as high as 1 μM) (Azorlosa *et al*., 1992), and Δ^9-THC may accumulate in fat tissues at levels that are up to 20-fold higher than those in normal tissues (Johansson *et al*., 1989). Although further studies are needed in order to clarify the activation mechanisms used by as well as the significance of phytocannabinoids in pathophysiologies, this is the first study to demonstrate that the phytocannabinoids, Δ^9-THC and CBD activate the enzymatic activity of human calpain-1.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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