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Inhibition of store-operated Ca^{2+} entry channels and K^+ channels by caffeic acid phenethylester in T lymphocytes

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ABSTRACT

The increase of cytoplasmic Ca^{2+} concentration ($\Delta[Ca^{2+}]_c$) in response to antigenic stimulation is a critical step of signals activating immune responses. In addition, the voltage-gated K^+ channels (Kv) in T lymphocytes draw attention as an effective target of immune-modulation. Caffeic acid phenethyl ester (CAPE), an active component of propolis, shows strong anti-inflammatory effects and T cell suppression. Although various mechanisms have been suggested for the action of CAPE, the effects of CAPE on intracellular Ca^{2+} signaling and ion channels are unknown. Here we investigated the effects of CAPE on $\Delta[Ca^{2+}]_c$, Ca^{2+}-release activated Ca^{2+} current (I_{CRAC}), and Kv current (I_{Kv}) in Jurkat T cells, and on Ca^{2+}-activated K^+ channel current (I_{SK4}) overexpressed in HEK-293 cells. I_{CRAC} was induced by dialyzing T cells and Orai1/STIM1 overexpressing HEK293 cells with $InsP_3$/BAPTA-containing pipette solution. CAPE concentration-dependently decreased both T cell receptor (CD3)- and thapsigargin-induced $\Delta[Ca^{2+}]_c$. The phosphorylation of PLCγ_1 by CD3 stimulation was not affected by CAPE. I_{CRAC} was almost completely blocked by 25 μM CAPE. CAPE also inhibited the I_{Kv} and I_{SK4}. Albeit the strong inhibition of Ca^{2+} influx via CRAC, the suppression of IL-2 secretion by CAPE was similarly observed in human peripheral T cells when the CRAC pathway was circumvented by ionomycin. Although the unspecific inhibition of ion channels by CAPE suggested an intriguing mechanism, the effects of CAPE on signaling pathways other than I_{CRAC} seem to play dominant roles in the immunomodulation by CAPE.

1. Introduction

Polyphenols are critical antioxidants in our diet and draw attention due to their potential therapeutic and health promoting effects. In addition to their antioxidant effects, it has been consistently proposed that polyphenols exert their biological actions by interacting with and regulating various enzymes such as telomerase, cyclooxygenase, and lipoxygenase (D'Archivio et al., 2007; Fraga, 2007). Also, because of their lipophilic structure, interactions with biomembranes and membrane proteins have been presumed. However, there are still rare investigation of the effects of polyphenols on ion channels and transporters.

Polyphenols are divided into several classes according to the number of phenol rings and structural components; flavonoids, phenolic acids, phenolic alcohols, stilbenes and lignans (Fraga, 2007). Caffeic acid (CA) is the most abundant phenolic acid in dietary substances. A well-known derivative of CA is caffeic acid phenethyl ester (CAPE) that is recognized as the active component of propolis. CAPE has antiviral, anti-inflammatory, antioxidant, and immunomodulatory properties (Huang et al., 1976; Chiao et al., 1995; Mirzoeva and Calder, 1996; Michaluart et al., 1999; Fitzpatrick et al., 2001). In accordance with the above effects, it has been demonstrated that CAPE is a potent and specific inhibitor of NF-κB (Natarajan et al., 1996), lipid peroxidation (Laranjinha et al., 1995; Fitzpatrick et al., 2001) and lipoxygenase (Sud'ina et al., 1993). Also, CAPE inhibits the transcriptional activity of the cyclooxygenase type 2 (COX-2) gene in epithelial cells (Michaluart et al., 1999), inducible nitric-oxide synthase gene expression in macrophage cell lines (Song et al., 2002; Nagaoka et al., 2003), suppression of eicosanoid synthesis and arachidonic acid release (Mirzoeva and Calder, 1996; Michaluart et al., 1999). However, it is still not clear how the various effects are caused by single compound like CAPE. In this respect, it was

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Table 1
Summary of the decrease of fura-2 fluorescence by CAPE.

CAPE (μM)	Number (n)	$F_{control}/F_{CAPE}$ at 340 nm	$F_{control}/F_{CAPE}$ at 380 nm
10	14	1.10 ± 0.004	1.03 ± 0.017
25	10	1.33 ± 0.010	1.06 ± 0.010
50	6	1.75 ± 0.028	1.14 ± 0.010

suggested that the target site of CAPE might be an early step of signaling pathways in immunocytes.

In lymphocytes, CAPE is a potent inhibitor of mitogen-induced T cell proliferation and lymphokine production (Ansorge et al., 2003). The inhibition of NF-κB and *N*uclear *F*actor of *A*ctivated *T* cells (NFAT) has been suggested as the mechanism of T cell inhibition (Natarajan et al., 1996; Marquez et al., 2004). In various immunocytes including T cells, NFAT is a critical transcription factor activated by calcineurin-dependent dephosphorylation. Calcineurin is Ca^{2+}/calmodulin-dependent phosphatase, and the signaling steps proximal to the calcineurin-dependent NFAT dephosphorylation was suggested as the site of inhibitory action by CAPE (Natarajan et al., 1996; Marquez et al., 2004). However, direct investigation of whether CAPE affects the calcium signaling in T cells has not been performed yet.

Stimulation of T cell receptors (TCR) and co-receptors typically induces phospholipase C-γ1 (PLCγ$_1$)-mediated Ca^{2+} release from intracellular stores. The depletion of Ca^{2+} stores evokes sustained Ca^{2+} influx (*s*tore-*o*perated *C*a^{2+} *e*ntry, SOCE) that is believed to be critical for the calcineurin activation and subsequent dephosphorylation of NFAT (Quintana et al., 2005; Feske, 2007). Among different types of ion channels activated in the process of SOCE, a specific group of highly Ca^{2+}-selective channels (*C*a^{2+}-*r*elease *a*ctivated *C*a^{2+} channels; CRAC) showing distinctive inwardly rectifying current–voltage relation are regarded as the principal one. The Ca^{2+} current via CRAC (I_{CRAC}) was firstly recorded in mast cells and intense investigation has been done in T cells (Hoth and Penner, 1992; Parekh and Putney, 2005). While the molecular identity of CRAC has been a long dispute, recent findings finally demonstrated that Orai1 (CRACM1) is the Ca^{2+} conducting pore unit in plasma membrane, and that STIM1 is the sensor protein in ER membrane (Feske, 2007). The case of immune-suppressed patients having defective mutations of Orai1 demonstrated the importance of CRAC (Feske et al., 2006).

In addition to CRAC, K^+ channels in T cells also are drawing attention as therapeutic targets for immunomodulation (Chandy et al., 2004; Beeton et al., 2006). Voltage-gated K^+ channel (Kv1.3) and intermediate-conductance Ca^{2+}-activated K^+ channel (SK4, IKCa1) are representative K^+ channels expressed in human T cells. These K^+ channels are thought to play roles in the immunological synapse formation and provide electrical driving forces for the Ca^{2+} influx through CRAC (Chandy et al., 2004; Feske, 2007).

In the present study we investigated the effects of CAPE on the Ca^{2+} signaling and PLCγ1 phosphorylation in Jurkat T cells. Also, the effects of CAPE and CA on I_{CRAC} and K^+ channels (Kv and SK4) cells were investigated. The potent inhibitory effects on these key ion channels in human lymphocytes suggested that the anti-inflammatory and immunomodulatory actions of CAPE might not be circumscribed to the inhibition of enzymes but also extend to the ion channels.

2. Materials and methods

2.1. Cell preparation and culture conditions

Jurkat T cells (clone E6-1) were purchased from the American type Culture Collection (Manassas, VA 20108, USA) and were grown in RPMI 1640 media (Gibco, Grand Island, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Gibco), 1 mM sodium pyruvate (Gibco), and 1% penicillin/streptomycin (Gibco). All cells were incubated at 37 °C in a humidified atmosphere (95% air/5% CO_2).

2.2. HEK293 cells expressing Orai1/STIM1

For expression of proteins in HEK 293 cells, cells were grown in 100 mm dishes to 80% confluence. HEK293 cells were transfected with an expression vector carrying the hOrai1 or hSTIM1 genes cloned into the N-terminal portion of enhanced green fluorescent protein (EGFP) by using a kit (Fugene 6, Roche, Germany) according to the product's manual. The cloned genes of hOrai1 and hSTIM1 were kind gifts from Dr. Tomohiro Kurosaki, RIKEN, Japan. The amount of DNA was 1 μg. Transfected cells were grown in Dulbecco's Modified Eagle's medium (Gibco Grand Island, NY) supplemented with 10% (v/v) fetal bovine serum (Hyclone, Logan, USA), and 1% penicillin/streptomycin (Gibco) at 37 °C in 90% air/10% CO_2. For measurement of I_{CRAC}, the cells were replated 24 h after the transfection.

2.3. $[Ca^{2+}]_c$ measurement

The measurement of $[Ca^{2+}]_c$ was done in HEPES-buffered physiological salt solution (PSS) containing 145 mM NaCl, 3.6 mM KCl, 10 mM HEPES, 1.3 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM D-glucose and

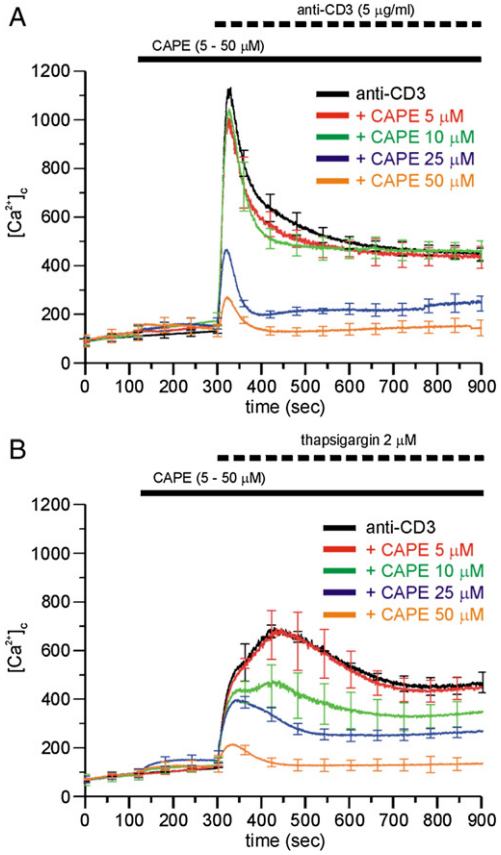

Fig. 1. Inhibition of agonist- and thapsigargin-induced increase of $[Ca^{2+}]_c$ by CAPE. Application of anti-CD3 antibody (anti-CD3, 5 μg/ml) induced a transient increase of $[Ca^{2+}]_c$ that reversed to a steady-state higher than resting control (A). In (B), application of thapsigargin (2 μM) induced slower increase of $[Ca^{2+}]_c$ that was also partially reversed to a plateau level. Pretreatment with CAPE (5–50 μM) concentration-dependently decreased the effects of anti-CD3 and thapsigargin on $[Ca^{2+}]_c$ where the decrease of plateau phase was more prominent. The traces were coded by different colors and reflect the mean S.E.M. of five experiments for each concentration of CAPE. Note that the application of CAPE alone induced a slight increase of $[Ca^{2+}]_c$ at relatively high concentrations.