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Piperine, a component of black pepper, decreases eugenol-induced cAMP and calcium levels in non-chemosensory 3T3-L1 cells

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ABSTRACT

This study investigated the effects of an ethanol extract of black pepper and its constituent, piperine, on odorant-induced signal transduction in non-chemosensory cells. An ethanol extract of black pepper decreased eugenol-induced cAMP and calcium levels in preadipocyte 3T3-L1 cells with no toxicity. Phosphorylation of CREB (cAMP response element-binding protein) was down-regulated by the black pepper extract. The concentration (133.8 mg/g) and retention time (5.5 min) of piperine in the ethanol extract were quantified using UPLC–MS/MS. Pretreatment with piperine decreased eugenol-induced cAMP and calcium levels in 3T3-L1 cells. Piperine also decreased the phosphorylation of CREB, which is up-regulated by eugenol. These results suggest that piperine inhibits the eugenol-induced signal transduction pathway through modulation of cAMP and calcium levels and phosphorylation of CREB in non-chemosensory cells.

1. Introduction

Black pepper (*Piper nigrum*, PN), of the *Piperaceae* family, is one of the most widely used condiments in the world. In addition to its use as a spice, black pepper has been used in conventional medicines to treat pain, flu, muscle aches, and rheumatism as well as to stimulate appetite [1]. Black pepper contains various active phytochemicals such as alkaloids, terpenes, flavones, and steroids, and many studies have reported their physiological effects in human and animals [1,2]. Although the exact amount of piperine, a main alkaloid in black pepper, varies owing to different methods of extraction and analysis, piperine comprises approximately 2–30% of dried black pepper [3,4]. After piperine (Fig. 2B) was first separated and purified by Oersted in 1820 [5], numerous studies have demonstrated its potential health benefits [1,2]. Piperine is an anti-inflammatory molecule inhibiting the production of prostaglandin E2 and nitric oxide in RAW264.7 cells [6]. It also suppresses stress-induced behavior by increasing serotonin and brain-derived neurotropic factors [7]. In high-fat diet-induced obese mice, piperine was shown to activate AMP-activated protein kinase and PPARδ and attenuate HFD-induced obesity [8]. Inhibition of ERK1/2 signaling by piperine reduced SREBP-1 and FAS expression in breast cancer cells, suggesting that it could be used as an antitumor agent to prevent or treat human breast cancer [9]. Recently, this possibility was further supported by data showing that piperine induced apoptosis of melanoma cells by decreasing XIAP, Bid, and caspase-3 [10]. However, the physiological roles of black pepper and piperine have not yet been elucidated in the odorant-induced signal transduction (OST) pathway in non-chemosensory cells.

Olfaction is the sense of smell. The perception of odor is important for survival and is required to select food and mates as well as to respond to the fear of predators. However, recent reports demonstrate that OST plays not only a role in olfaction but also other physiological roles in non-chemosensory tissues [11]. Ectopic expressions of olfactory receptors in sperm, kidney, and muscle were involved in the chemotaxis of sperm, glomerular filtration rate, and muscle regeneration, respectively [12–14]. The ectopic expression was also supported by recent reports showing that olfactory receptors were expressed in fat tissue of diet-induced obese mice and eugenol receptor (mOR-EG, Olfr73, MOR174-9) was expressed in 3T3-L1 cells [15,16]. However, their physiological roles and regulations in fat tissue and non-chemosensory cells are

Abbreviations: CREB, cAMP response element-binding protein; OST, odorant-induced signal transduction; PNF, *Piper nigrum* fructus

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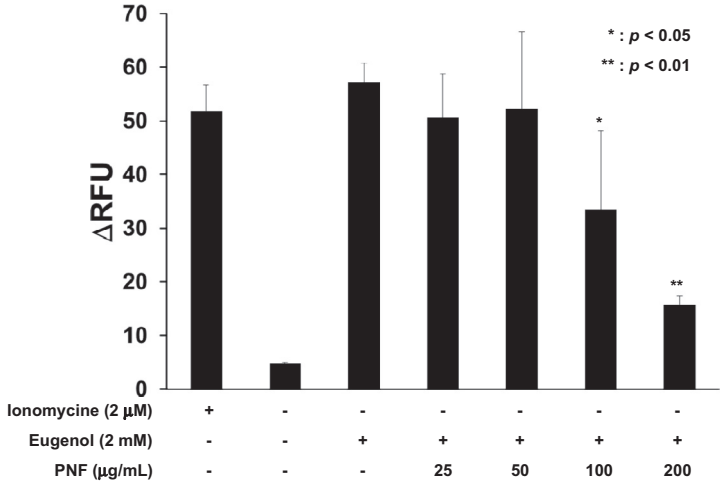

Fig. 1A. Changes in Ca^{2+} level induced by eugenol in 3T3-L1 cells after pretreatment with an ethanol extract of black pepper for 30 min. Ionomycin (2 μM) was used as a positive control and DMSO (1%) was used as a negative control. The data are shown as means ± SD (n = 3 for 25 and 200 μg/mL, n = 5 for 50 and 100 μg/mL). $^{**}p < 0.01$. ΔRFU, change in relative fluorescence unit.

largely unknown. Interestingly, the OST pathway in these non-chemosensory tissues shares the same mechanism, where odorants stimulate signals by binding to olfactory receptors, and cAMP and Ca^{2+} act as second messengers to relay the signal cascade in order to achieve olfactory perception and other physiological effects in neuronal and non-chemosensory cells, respectively [12–14].

In this study, we investigated the effects of black pepper and its constituent, piperine, on the OST pathway in non-chemosensory 3T3-L1 cells. When an odorant was used to stimulate non-chemosensory cells, we observed inhibitory effects of the ethanol extract of black pepper and piperine through regulation of Ca^{2+} and cAMP levels.

2. Materials and methods

2.1. Plant material

Ethanol extract of *P. nigrum* fructus (PNF) was obtained from the Korea plant extract bank at the Korea Research Institute of Bioscience and Biotechnology (KRIBB, Daejeon, Republic of Korea). The powder was dried at room temperature and then dissolved in 95% ethanol (v/v).

2.2. Reagents and antibodies

Piperine and eugenol were purchased from Sigma (St. Louis, MO, USA). The Ca^{2+} assay kit was obtained from Molecular Devices (Sunnyvale, CA, USA). The cAMP assay kit was purchased from Enzo Life Sciences (Plymouth Meeting, PA, USA). Antibodies against phospho-CREB and lamin B1 were purchased from Cell Signaling Technology (Beverly, MA, USA).

2.3. Cell culture and viability assay

3T3-L1 cells were obtained from American Type Culture Collection (Manassas, VA, USA) and cultured in DMEM (high glucose), which contained 10% FBS and 1× antibiotic–antimycotic solution (WelGENE Inc., Daegu, Republic of Korea). The cells were incubated at 37 °C in the presence of 5% CO_2. The viability of cells was determined using the Cell Proliferation Reagent WST-1 (Roche Diagnostics, Mannheim, Germany). Cells at a concentration of 4×10^4 cells/well in 500 μl culture medium were seeded in 24-well plate. Incubated cells for 6 h at 37 °C and 5% CO2 and washed it with serum-free medium, then added 500 μl serum-free medium containing the extract or piperine. After 24 h, added 20 μl/well Cell Proliferation Reagent WST-1 and incubate the cells for 1 h. Shake

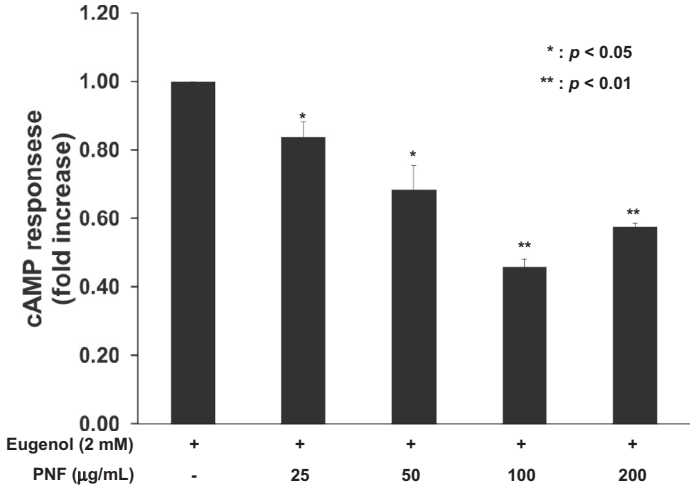

Fig. 1B. Changes in cAMP level induced by eugenol in 3T3L1 cells after pretreatment with an ethanol extract of black pepper for 30 min. The final concentration of DMSO in all samples was 1%. The data are shown as the mean ± SD (n = 3). ΔRFU, change in relative fluorescence unit.