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Short communications

Mechanisms and consequences of carnosine-induced activation of intestinal epithelial cells

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ABSTRACT

The molecular basis for the carnosine-induced activation of intestinal epithelial cells was studied and subsequently we focused on whether carnosine stimulates a brain–gut interaction. To assess this, we investigated changes in intestinal epithelial cells induced by carnosine. Our results showed that carnosine activated Caco-2 cells, resulting in the secretion of various factors (including neurotrophic factors), and leading to the induction of neurite growth in SY-SY5Y cells. We then conducted DNA microarray analysis to reveal global changes in Caco-2 cells via treatment with carnosine. The expression of 745 genes significantly changed upon carnosine treatment. Furthermore, cluster analysis showed that several of these genes were related to secretory proteins, membrane protein/transporters, and calcium channel/transport protein. Some of these genes would explain the mechanism of carnosine action, especially considering stimulation of the brain–gut interaction.

1. Introduction

Chicken breast extract (CBEX) is a commercially available functional food rich in carnosine (β-alanyl-L-histidine) and its derivative anserine (β-alanyl-1-methyl-L-histidine). Supplementation with CBEX is known to improve relatively high intensity endurance performance in humans (Maemura et al., 2006), and to induce antidepressant-like activity in rats (Tomonaga et al., 2008). To clarify the molecular mechanisms of CBEX functions, many researchers have focused on carnosine, one of major ingredients of CBEX. Carnosine is now recognized as one of the remarkable food ingredients.

Carnosine is a multifunctional dipeptide with many roles including buffering (Davey, 1960), free radical scavenging (Chasovnikova, Formazyuk, Sergienko, Boldyrev, & Severin, 1990; Samaranayaka & Li-Chan, 2011), enzyme regulation (Johnson & Aldstadt, 1984), and sarcoplasmic reticulum Ca^{2+} regulation (Batrukova & Rubtsov, 1997; Culbertson, Kreider, Greenwood, & Cooke, 2010). Along with its many roles, carnosine is widely distributed in tissues and exists at particularly high concentrations in muscles and the brain (De Marchis et al., 1997). Due to these functionalities, carnosine can be used to prevent and treat various diseases such as diabetes, neurodegenerative diseases, diseases of the sensory organs, metabolic syndrome, and cancers (Budzeń & Rymaszewska,

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2013). Carnosine is potentially beneficial in the treatment of Alzheimer's disease (AD) because of its free-radical scavenging, metal-chelating activities as well as its ability to suppress inflammation and cerebrovascular abnormalities (Corona et al., 2011; Herculano et al., 2013).

In the present study, we attempted to determine the molecular basis for the carnosine-induced activation of intestinal epithelial cells, and then focused on molecules functioning in the brain–gut interaction. We used a human colon cancer-derived cell line, Caco-2, to clarify factors secreted from these cells that might function to activate the brain–gut interaction.

2. Materials and methods

2.1. Cell culture and reagents

We used a human colon cancer line, Caco-2, and a human neuroblastoma cell line, SH-SY5Y. Caco-2 and SH-SY5Y were cultured in Dulbecco's modified Eagle's medium (DMEM; Nissui, Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS; Life Technologies, Gaithersburg, MD, USA) at 37 °C in 5% CO_2, and carnosine was purchased from Wako (Osaka, Japan).

2.2. Differentiation of SH-SY5Y cells (Encinas et al., 2000; Jämsä, Hasslund, Cowburn, Bäckström, & Vasänge, 2004; Kou, Luchtman, & Song, 2008)

After SH-SY5Y cells were cultured in DMEM medium supplemented with 10% FSB for 1 d, cells were cultured in DMEM medium supplemented with 10% FBS and 10 μM retinoic acid (RA) for 5 d. After washing the cells with DMEM medium, cells were treated with 50 ng/mL of brain-derived neurotrophic factor (BDNF) and cultured in DMEM medium for an additional 6 d.

2.3. Quantitative reverse transcriptase polymerase chain reaction

RNA was prepared using the High Pure RNA Isolation kit (Roche Diagnostics GmbH, Mannheim, Germany), and cDNA was prepared using the ReverTra Ace (Toyobo, Osaka, Japan), according to the manufacturers' protocols. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was performed using the KAPA SYBR Fast qPCR Kit (KAPA Biosystems, Woburn, MA, USA) and Thermal Cycler Dice Real Time System TP-800 (TaKaRa, Shiga, Japan), as described previously (Fujiki et al., 2007). Samples were analyzed in triplicate, and gene expression levels were normalized to the corresponding β-actin level. Polymerase chain reaction primer sequences were shown in Table S1.

2.4. Quantitative evaluation of neurite growth

After SH-SY5Y cells were fixed with 4% paraformaldehyde for 30 min, they were washed 3 times with phosphate-buffered saline (PBS). Cells were then blocked with blocking buffer (1 × PBS, 1% bovine serum albumin, 5% FBS, 0.2% Triton X-100) for 1 h, and were stained with Milli-Mark FluoroPan Neuronal Marker (Merk Millipore, Billerica, MA, USA) at room temperature for 2 h. After cells were washed with PBS, neurite length was measured using IN Cell Analyzer 1000 (GE Healthcare, Amersham Place, UK).

2.5. ELISA

The concentration of BDNF secreted into the culture supernatant was measured using BDNF Emax Immunoassay system (Promega, Madison, WI, USA) according to the manufacturer's protocol.

2.6. DNA microarray (Miyahara et al., 2014)

Total RNA was isolated from Caco-2 cells using TRIzol Reagent (Life Technologies, Gaithersburg, MD, USA), and purified using the SV Total RNA Isolation System (Promega). RNA quality was checked using an Experion automated electrophoresis station (Bio-Rad Laboratories, Hercules, CA, USA).

cRNA was labeled using Low Input Quick Amp Labeling (Agilent Technologies, Santa Clara, CA, USA), and hybridized to DNA microarray (Human Whole Genome ver. 2.0, 4 × 44 K format, Agilent). Relative hybridization intensities and background hybridization values were calculated using Feature Extraction Software (Agilent).

To identify genes with altered expression, we calculated intensity-based Z-scores and ratios from the normalized signal intensities of each probe and then compared control and experimental samples (exposed to carnosine). We then established criteria for significantly up/down-regulated genes: up-regulated genes, Z-score ≥ 2.0 and ratio ≥ 1.5-fold; down-regulated genes: Z-score ≤ –2.0 and ratio ≤ 0.66-fold. To determine significantly over-represented gene ontology (GO) categories and significantly enriched pathways, we used tools and data provided by the Database for Annotation, Visualization, and Integrated Discovery (DAVID, http://david.abcc.ncifcrf.gov) (Huang, Sherman, & Lempicki, 2009a, 2009b). Results were generated from control versus carnosine-treated cells. We next generated a heat map using MeV software (Saeed et al., 2003), and a hierarchical clustering method was used to sort the genes. Color indicates the distance from the median of each row.

2.7. Statistical analysis

All results are expressed as mean ± standard error of mean. Statistical difference was determined by a two-sided Student's *t*-test, with $P < 0.05$ considered as statistically significant.

3. Results and discussion

3.1. Carnosine-activated Caco-2 cells secreted soluble factors that induced neurite growth in SH-SY5Y cells

Carnosine has so far been reported to have various functions including buffering, and antioxidation, among others. In the present study, we tried to clarify whether carnosine stimulates a brain–gut interaction through augmenting secretory neurotrophic factors from intestinal cells. Negative control