Analysis of the mechanism of the tight-junctional permeability increase by capsaicin treatment on the intestinal Caco-2 cells

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Abstract
In a previous experiment (Isoda et al., 2001), we showed that the tight-junctional (TJ) permeability increase in Caco-2 cells during capsaicin exposure was through binding of the capsaicin molecule to a capsaicin receptor-like protein. In the present study, we examined how actin, which modulates TJ permeability, is influenced by capsaicin. We showed that after treatment of the Caco-2 cells with capsaicin, the volume of F-actin decreased. Moreover, we also examined protein kinase C (PKC) and heat shock protein 47 (HSP47), which act as probable second messengers in causing TJ permeability increase. We showed that after capsaicin treatment, HSP47 was activated. However, PKC activity was the same in both control and treatment setups. These results suggest that, while PKC is not involved, it is highly possible that HSP47 plays a role in TJ permeability increase in intestinal Caco-2 cells exposed to capsaicin.

Abbreviations: BSA – bovine serum albumin; CHO cells – Chinese Hamster Ovary cells; DMEM – Dulbecco’s modified eagle’s medium; ER – endoplasmic reticulum; F-actin – filamentous actin; G-actin – globular actin; HSP47 – heat shock protein; LDH – lactic dehydrogenase; PBS – phosphate-buffered saline; PKC – protein kinase C; TER – transepithelial electrical resistance.

Introduction
In our previous study (Isoda et al., 2001), the influence of capsaicin processing on human intestinal cell line Caco-2 was examined by measuring transepithelial electrical resistance (TER). The permeability of the Caco-2 cell monolayer by measuring the TER value is known to be correlated with a change in the paracellular permeability of the cell monolayer (Hashimoto et al., 1997). There was an increase in permeability at high concentration (200 to 500 µM) of capsaicin, and the effect was inhibited by pretreatment of capsazepine, which is a competitive antagonist of the vanilloid receptor 1 (VR1). We also determined the expression of the VR1-like protein on Caco-2 cells in a time-dependent manner by western blotting using VR1 antiserum. These results show that the permeability increase by capsaicin was through binding of capsaicin to VR1-like protein of Caco-2 cells. In these consecutive phenomena, LDH (lactic dehydrogenase) activity as well as changes in the intracellular Ca2+ were determined to know whether or not capsaicin affected TER activity through influence on the tight junction. LDH activity and the increase in the intracellular Ca2+ were considerably high at capsaicin concentration of 200 µM. However, there were no remarkably high LDH activity in Caco-2 cells at concentrations above 300 and 400 µM of capsaicin. These results suggest that the decrease in TER value during capsaicin treatment was not due to cell monolayer damage, but rather, to the reversible opening of a paracellular route.

In this study, possible mechanisms involved in tight-junctional (TJ) permeability increase by cap-
Capsaicin were investigated. Tight junctions play a critical role in epithelial cell biology by forming a selectively permeable barrier in the spaces between epithelial cells and maintaining the cell surface compositional asymmetry, which is characteristic of this cell type. The TJ has been thoroughly characterized morphologically and physiologically; however, only in the last decade has the molecular configuration begun to be understood. The TJ contains at least nine peripheral and three integral membrane proteins, and several of these have been implicated in tumor suppression, growth regulation and signal transduction (Yap et al., 1998). TJ permeability increase is modulated through actin, which is a 43 KDa protein, and the majority of the isotype heterogeneity is located at the amino-terminal 30 amino acid. The amino-terminus of globular-actin (G-actin) is located at the periphery of the double-helix in filamentous-actin (F-actin). G-actin readily polymerizes under physiological conditions to form F-actin with the concomitant hydrolysis of ATP (Holmes et al., 1990; Rayment et al., 1993).

HSP (heat shock protein) 47 is a novel 47 KDa stress glycoprotein and its expression is highly tissue- and cell-specific. It is likewise restricted to most phenotypically altered collagen-producing cells. Prior to secretion, procollagen molecules are correctly folded into triple helices in the endoplasmic reticulum (ER) where HSP47 specifically associates with procollagen during its folding and/or modification processes and is thought to function as a collagen-specific molecular chaperone (Mohammed et al., 2000; Koide et al., 1999). It was demonstrated that the synthesis of HSP47 paralleled that of types I and IV collagen (Nagata et al., 1986, 1996). In recent studies, the expression of HSP47 was shown to be associated with increased staining of collagen types I, II and IV in an experimental model of interstitial fibrosis of the kidney (Cheng et al., 1998; Moriyama et al., 1998). The expression of collagen-binding HSP47 with various proteins implicated in phenotypic modulation (actin filaments and vimentin) and fibrosis (type I and type III collagens) was examined in control and cisplatin-treated kidneys (Razzaque et al., 1999).

Protein kinase C (PKC), one of the most important proteins in signal transduction, is involved in cell proliferation. It was suggested that PKC is connected with the F-actin cytoskeleton (Savala et al., 1998). Further, it has reported that PKC activation was accompanied with the translocation of this enzyme from the cytosol to the plasma membrane (Hashimoto et al., 1997).

From these results, it is assumed that PKC is involved with tight-junctional permeability increase.

In this study, we investigated the possibility that capsaicin triggers cytoskeletal reorganization of the actin filaments and activation of HSP47 and PKC, which are implicated in cytoskeletal reorganization of the actin filaments. It is done to help elucidate the pathway by which capsaicin increases TJ permeability.

Materials and methods

Materials

Capsaicin, Dulbecco’s modified Eagle’s medium (DMEM), penicillin-streptomycin MDCB302, 4-Methylumbellifer β-D-galactoside (M1633) and fetal calf serum (FCS) were obtained from Sigma (U.S.A.). Non-essential amino acid was purchased from Cosmo Bio Co. Ltd. (Tokyo, Japan). Bovine serum albumin (BSA), kanamycin, L-glutamine, rhodamine-phalloidin (R-415) were purchased from Wako Pure Chemical Industries (Osaka, Japan). The Pep Tag Protein Assay Kit and lysis buffer were obtained from Promega (U.S.A.).

Cell culture

The Caco-2 cells were maintained in DMEM supplemented with 10% FCS, 1% penicillin-streptomycin, and 1% non-essential amino acids. They were incubated in an atmosphere of 5% CO2 at 37 °C. The cells were passaged at a split ratio of 4 to 8 every 3 or 4 days. For determination of volume of F-actin, cells were seeded on a chamber slide (LabTek, U.S.A.) at a density of 2 × 10^5 cells per slide. For the PKC assay, cells were seeded on Petri plates at a density of 1 × 10^6 cells per well.

The HSP47-transformed Chinese Hamster Ovary cells (CHO cells) were maintained in MDCB302 (5.5 g L^-1) and DMEM (4.75 g L^-1) supplemented with L-glutamine (0.3 g L^-1), NaHCO3 (1.27 g L^-1), kanamycin (0.1 g L^-1), and incubated in an atmosphere of 5% CO2 at 37 °C. The cells were passaged at a split ratio of 4 to 8 every 3 or 4 days. For determination of the HSP47 promoter activated by capsaicin, cells were seeded onto 96-well plates at a density of 1 × 10^4 cells/well.