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Opposing regulation of histamine-induced calcium signaling by sodium selenite and ebselen *via* alterations of thiol redox status

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ABSTRACT

Elevated blood histamine plays a role in the pathogenesis of atherosclerosis. Calcium signaling mediates histamine action in endothelial cells. Selenium (Se) is a dietary essential trace element for humans. Se compounds in different oxidation states were found to exhibit an opposing effect on the histamine-induced calcium signaling in the ECV304 cell line. When Se in the form of sodium selenite was added in the cell culture, the reactivity of the histamine H_1-receptor was increased as reported in our previous paper. We here show that as a culture supplement, sodium selenite enhanced the activity of selenoprotein thioredoxin reductase (TrxR) and the calcium response to histamine stimulation, which were reversed by treating the cells with gold thioglucose, a nucleophilic drug that selectively modifies thiolate/selenolate groups. Sodium selenite most likely caused a reductive shift in the thiol/disulfide redox balance through increasing TrxR activity. In contrast, when the cells were treated with Se in the form of ebselen, a thiol oxidant with peroxidase-like activity, histamine-induced calcium release and calcium entry were significantly suppressed. This effect appeared related to the thiol-directed modification rather than the peroxidase-like activity of ebselen, because this inhibitory effect was not replicated by increasing cellular peroxidase activity. Thus, the opposing effects of sodium selenite and ebselen on histamine-induced calcium signaling are achieved, at least in part, through their opposite actions in modulating the thiol/disulfide redox state.

1. Introduction

The role of elevated blood histamine in the pathogenesis of atherosclerosis has received considerable attention. Histamine is a low-molecular-weight amine, synthesized from L-histidine exclusively by histamine decarboxylase. Histamine levels were found to increase in stable coronary artery disease and in acute coronary syndrome (Clejan et al., 2002). The effects of histamine on several receptor pathways are believed to be involved in atherosclerosis (Tanimoto et al., 2006). For instance, histamine is able to induce expression of P-selectin, leading to recruitment of rolling leukocytes (Asako et al., 1994; Jones et al., 1993). The leukocyte count affects coronary heart disease (Madjid et al., 2004). Atherosclerosis is characterized by unusual growth of vascular smooth muscle cells in the intima. Histamine promotes DNA synthesis and Pro-matrix metalloproteinase 1 expression (Satoh et al., 1994). Both histamine H_1-receptor and histamine H_2-receptor antagonists were found to protect the intimal thickening of mice femoral arteries (Miyazawa et al., 1998).

Binding of histamine to histamine receptors on endothelial cells results in an increase in cytosolic calcium concentration (Zheng et al., 2005). Cellular calcium content has an important role in endothelial cell functions, and is required to be strictly controlled because prolonged elevation of cytosolic calcium in the μM range is deleterious to cells. One of the many factors that regulate cellular calcium content is intracellular thiol/disulfide redox status (Kuo et al., 2003). A correlation between thiol-disulfide oxidoreduction and regulation of calcium signaling has been described for certain molecules, including calcium-ATPases, Na^+/Ca^{2+} exchanger, calmodulin (Barnes et al., 2000; Goldhaber, 1996; Yin et al., 2000) and histamine H_1-receptor (Donaldson and Hill, 1987). The thiol/disulfide state of these proteins is the primary target for the cellular redox system to regulate calcium signaling.

Endothelial cells are known to express histamine H_1-receptor, whose up-regulated expression is involved in the initiation and progression of cardiovascular diseases (Takagishi et al., 1995). This receptor mediates histamine-induced calcium signaling *via* the following reaction cascade: histamine H_1-receptor → phospholipase C (PLC) → phosphatidylinositol-4,5-bisphosphate (PIP_2) → inositol 1,4,5-trisphosphate (IP_3) → IP_3 receptor → calcium signaling. Histamine H_1-receptor contains thiol-disulfide groups (Leurs et al., 1990) and its agonist affinity was significantly enhanced by disulfide reducing agent dithiothreitol (DTT) (Dickenson and Hill, 1994). PLC-alpha is a member of Trp–Cys–Gly–His–Cys–Lys motif-containing proteins including protein disulfide isomerase (PDI) and thioredoxin (Hirano et al., 1994) that are substrates of thioredoxin reductase (TrxR) (Holmgren, 1985; Lundstrom and Holmgren, 1990), a major

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selenoprotein in human endothelial cells (Anema et al., 1999). Our previous study showed that the addition of sodium selenite into the cell culture medium caused an increase in cellular TrxR activity and histamine-induced IP_3 response (Zheng et al., 2005), suggesting that the effect of selenium (Se) on IP_3-related calcium signaling can be mediated by increasing TrxR activity.

Se is a dietary essential trace element for humans, which is required in small amounts. Se is specifically incorporated into proteins in the form of selenocysteine and non-specifically incorporated into proteins as selenomethionine in the place of methionine. Recommended Dietary Allowances (RDAs) for adult are 55–70 μg Se/day. At higher concentrations, Se compounds can be cytotoxic (Drake, 2006). For a long time, Se supplementation, as sodium selenate, sodium selenite, and organic forms of selenium, was widely promoted as a beneficial antioxidant. However, recent reports indicated that most trials, conducted in selenium-replete populations, found no evidence of cardiovascular protection (Navas-Acien et al., 2008). We thus speculated that since Se commonly exists in the −2, 0, +4, and +6 oxidation states, their effects could be different. It is of considerable interest to know whether selenium in different oxidation states may exert a distinct effect on histamine-induced calcium signaling.

We here report opposing effects of two selenium compounds, sodium selenite and ebselen in different oxidation states, on histamine H_1-receptor-mediated calcium signaling. Our results suggest that selenium in low to high oxidation states may have different effects on thiol/disulfide balance, which should be considered when analyzing the roles of selenium compounds in health and disease.

2. Materials and methods

2.1. Materials

Fura-2/AM and 2,7-dihydrodichlorofluorescin diacetate (H_2DCF-DA) was obtained from Molecular Probes, Carlsbad, CA, USA. Dulbecco's modified eagle medium (DMEM) was obtained from Gibco, Grand Island, NY, USA. Guanidine hydrochloride was purchased from Bebco, Kansas, AZ, USA. Sodium selenite (Na_2SeO_3) was obtained from Daiichi, Pharmaceutical Co., Ltd., Tokyo, Japan. Ebselen [2-phenyl-1,2-benziso-selenazol-3(2H)-one] was a product of Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan, and dissolved in fresh dimethyl sulfoxide (DMSO) just before use. The final concentration of DMSO in the medium never exceeded 0.1%. Histamine dihydrochloride, gold thioglucose, 5, 5′-Dithiobis(2-nitrobenzoic acid) (DTNB) and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma, St Louis, MO, USA. All other reagents were of analytic grade.

2.2. Cell culture

ECV304 cells, a spontaneously transformed immortal endothelial cell line, were grown in DMEM supplemented with 10% calf serum, 1 mg/ml D-glucose, 100 μg/ml streptomycin and 100 U/ml penicillin. The selenium content of the basal medium (without calf serum) was 0.35 nM (Lewin et al., 2002). The cells cultured in such medium were referred to as Se^0 cells. To study the effect of sodium selenite on the calcium signaling, the cells were grown under the same conditions in the media supplemented with 1 μM sodium selenite for 3–5 days, and were referred to as Se^+ cells. Under the experimental conditions, no specific side effects were observed in Se^+ cells. Before the measurements of cellular calcium, the cells were planted in a glass bottom dish and incubated at 37 °C overnight in DMEM. To examine the effect of gold thioglucose or ebselen on calcium signaling, the cells were pre-incubated with gold thioglucose or ebselen for 30 min at 37 °C before being stimulated with 1 mM H_2O_2 or 0.1 mM histamine. In view of the fact that ebselen, especially at concentrations of 20 and 40 μM, prevented ischemia-induced cytotoxicity (Gabryel and Malecki, 2006), at concentrations of 100 μM efficiently attenuated oxidative stress-induced neuronal cell death (Yoshizumi et al., 2002), and up to 100 μM had no effect on histamine release (Suzuki et al., 2003), we thus pretreated cells with 10–60 μM ebselen. Under these conditions, no direct toxic effects of ebselen were observed in our target cells.

2.3. Microscopic measurements of intracellular calcium concentration

The cells planted in the glass-bottomed dishes (2×10^5 cells) were incubated with 1.0 μM fura-2/AM at 37 °C for 40 min. In order to remove extracellular free fura-2/AM and sodium selenite, the cells were then washed four times either with a calcium buffer that was composed of 10 mM HEPES, 145 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$ (pH 7.4), or with a nominally calcium-free buffer, in which 2.5 mM $CaCl_2$ was replaced by 1.5 mM EGTA. Then 1.0 ml of a desired buffer was added into the dish and incubated at 37 °C for 10 min to allow for de-esterification of fura-2/AM. The attached cells on the bottom glass were excited by light from a mercury lamp alternately at 340 or 380 nm. The fluorescence images were taken every 20 s at an emission of 510 nm for 100 ms on an Olympus IX-71 inverted microscope equipped with an AquaCosmos Microscopic Image Acquisition and Analysis System provided by Hamamatsu Photonics K.K. (Hamamatsu City, Japan). The digitized fluorescence ratio (F_{340}/F_{380}) images of the cells and the kinetic change of the ratio in each cell were obtained and processed online on a PC computer.

Although the fluorescence ratio of F_{340} to F_{380} is not simply equivalent to the absolute value of cytoplasmic free calcium concentration, it can well represent the calcium concentration in fura-2 loaded cells. The cytoplasmic calcium concentration measured as the ratio of F_{340} to F_{380} in different measurements can be well compared as long as the baseline of the ratio was close enough.

2.4. Measurements of TrxR activity and protein concentration

Cells were harvested, and suspended in the cell lysis buffer which was composed of 50 mM potassium phosphate, pH 7.5, 1% Triton X-100, 2 mM EDTA and 1 mM PMSF. The cell suspension was placed in an ice bath and sonicated. The lysed cells were then heated at 65 °C for 10 min and spun at 13,000 rpm (11,000×g) for 30 min. The cleared supernatant was used as crude cell extracts, in which TrxR activity was measured using DTNB as substrate (Hill et al., 1997), and protein levels were determined by Bio-Rad Protein Assay system (Bio-Rad Laboratories, Hercules, CA, USA). The activity of TrxR was expressed as increases in absorption at 412 nm per mg of total protein in the crude cell extracts.

2.5. Measurements of intracellular reactive oxygen species generation in ECV304 cells

Intracellular accumulation of reactive oxygen species was detected by fluorescence microscopy (Olympus IX-71, Tokyo, Japan) and by a fluorescence spectrometer (F96, Shanghai Lengguang Technology Co., Ltd., Shanghai, China) using a reactive oxygen species-sensitive fluorescent probe, H_2DCF-DA. ECV304 cells were seeded at 1×10^5 cells/ml in 24-well plates, and cultured for 2 days. Before measurements of cellular reactive oxygen species by fluorescence microscopy, the cells were washed twice with PBS and loaded with 1 μM H_2DCF-DA in Hanks' Balanced Salt Solution (HBSS) buffer for 5 min at 37 °C. The fluorescent DCF was detected at the excitation and emission wavelengths of 488 and 525 nm, respectively. Before measurements of cellular reactive oxygen species by the fluorescence spectrometer, the cells were washed with PBS, and detached with 0.25% trypsin − 0.02% EDTA in PBS. The harvested cells were washed twice with PBS and loaded with 0.25 μM H_2DCF-DA for 5 min at 37 °C. The fluorescence intensity of DCF was measured and recorded at excitation and emission wavelengths of 488 and 525 nm, respectively.