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Research report

Carbonyl stress-induced 5-hydroxytriptamine secretion from RIN-14B, rat pancreatic islet tumor cells, via the activation of transient receptor potential ankyrin 1

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ABSTRACT

Methylglyoxal (MG), a highly reactive dicarbonyl substance, is known as an endogenous carbonyl stress-inducing substance related to various disease states. Irritable bowel syndrome (IBS) is one of the most frequently encountered gastrointestinal disorders and MG is considered to be its causal substance. An increased serum 5-hydroxytryptamine (5-HT) level is related to IBS symptoms and the majority of 5-HT originates from enterochromaffin (EC) cells in the intestine. Here we examine the mechanisms of MG-induced 5-HT secretion using RIN–14B cells derived from a rat pancreatic islet tumor since these cells are used as a model for EC cells. MG increased the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and 5-HT secretion, both of which were inhibited by the removal of extracellular Ca^{2+} and specific transient receptor potential ankyrin 1 (TRPA1) antagonists. MG elicited an inward current under voltage-clamped conditions. Prior application of MG evoked reciprocal suppression of subsequent $[Ca^{2+}]_i$ responses to allylisothiocyanate, a TRPA1 agonist, and vice versa. Glyoxal, an analog of MG, also evoked $[Ca^{2+}]_i$ and secretory responses but its potency was much lower than that of MG. The present results suggest that MG promotes 5-HT secretion through the activation of TRPA1 in RIN–14B cells. These results may indicate that TRPA1 is a promising target for the treatment of IBS and that the RIN–14B cell line is a useful model for investigation of IBS.

1. Introduction

Methylglyoxal (MG) is a highly reactive carbonyl intermediate produced by several pathways including glycolysis (Thornalley et al., 1999). In the stage of hyperglycemia, MG is abundantly produced and causes carbonyl stress resulting in many concomitant complications (Brownlee, 2001; Talukdar et al., 2009). In addition, MG produces advanced glycosylation end products by modifying proteins (Bierhaus and Nawroth, 2009). Glycation of the proteins is associated with the exacerbation mechanism of diseases, especially chronic clinical complications in conjunction with diabetes (Fosmark et al., 2006; Wang et al., 2007). Therefore, in the normal situation, biological tissues are protected from MG toxicity by the glyoxalase system (Allaman et al., 2015). Painful neuropathy occurs in chronic renal failure and diabetes, which involve elevated plasma MG levels (Lapolla et al., 2003; Han et al., 2007). Furthermore, MG is one of bacterial products of the anaerobic metabolism of sugar, and is suggested to change the balance of intestinal microflora causing IBS (Campbell et al., 2010).

IBS is a functional gastrointestinal disorder with various digestive organ symptoms such as diarrhea, constipation and abdominal pain (Longstreth et al., 2006). Alteration of the 5-HT metabolism in IBS patients has been reported (Cremon et al., 2011). A recent paper shows that the administration of MG to rats elicits symptoms of IBS such as higher fecal water contents and increases serum 5-HT values (Zhang et al., 2014). In addition to its pathophysiological significance, it has been reported that MG induces a number of biological actions such as changes of cellular $[Ca^{2+}]_i$ homeostasis in a variety of tissues. MG increases $[Ca^{2+}]_i$, resulting in promotion of insulin secretion in pancreatic β-cells (Cook et al., 1998; Cao et al., 2012). In rat arterial smooth muscles, MG inhibits noradrenalin-induced contraction by opening potassium channels due to an increase of $[Ca^{2+}]_i$ (Mukohda et al., 2009). Moreover, MG causes prolonged $[Ca^{2+}]_i$ increases and cytotoxic action in renal tubular cells (Jan et al., 2005) and mouse sensory neurons (Radu

Abbreviations: AITC, allylisothiocyanate; DMSO, dimethyl sulfoxide; EC cell, enterochromaffin cell; HPLC, high performance liquid chromatography; 5-HT, 5-hydroxytryptamine; IBS, irritable bowel syndrome; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; MG, methylglyoxal; TRPA1, transient receptor potential ankyrin 1.

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et al., 2012). However, the cellular mechanisms for MG-induced $[Ca^{2+}]_i$ increases are not fully understood.

TRPA1, a Ca^{2+}-permeable nonselective cation channel, is primarily expressed in sensory neurons, where its activation excites nociceptive neurons. It is activated by cold stimulation, some pungent compounds such as allylisothiocyanate (AITC) from mustard seeds, and environmental irritants (Chen and Hackos, 2015; Miura et al., 2013). TRPA1 is also expressed in secretary cells such as islet cells and is involved in insulin secretion (Cao et al., 2012; Numazawa et al., 2012). Since TRPA1 contributes to hyperalgesia during inflammation, the channel is considered to be a treatment target in the pathophysiological situation (Bautista et al., 2013).

In the present study, we measured $[Ca^{2+}]_i$ changes, membrane currents and 5-HT secretion induced by MG in RIN–14B cells, tumor cells from a rat pancreatic δ cell line (Braënstroëm et al., 1997). Since RIN–14B cells secrete 5-HT, they are considered to be a model for EC cells (Nozawa et al., 2009). We found that MG evoked $[Ca^{2+}]$ increases and 5-HT secretion in RIN–14B cells through the activation of TRPA1. These results suggest that MG-induced 5-HT secretion may be related to the etiology of IBS and its symptoms.

2. Experimental procedures

2.1. Chemicals

The following chemicals were used (vehicle, concentration for stock solution). Allylisothiocyanate (AITC) (dimethyl sulfoxide [DMSO], 1 M), methylglyoxal (DMSO, 1 M), and glyoxal solution (DMSO, 1 M) were from Nacalai, Tokyo, Japan. Capsazepine (DMSO, 0.05 M), HC-030031 (DMSO, 0.1 M), N-(3-Aminopropyl)-2-[(3-methylpheny l)methoxy]-N-(2-Thienylmethyl)benzamide hydrochloride (AMTB; DMSO, 0.05 M) were obtained from Sigma. A967079 (DMSO, 0.01 M) was from Focus Biomolecules (Pennsylvania, USA). Fluoxetin (DMSO, 20 mM) was purchased from Wako Pure Chemicals (Osaka, Japan). Other chemicals were purchased from Wako Pure Chemicals. These stock solutions were diluted more than 1000-fold with HEPES-buffered solution (in mM: 134 NaCl, 6 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 10 HEPES and 10 glucose, pH 7.4). We used 0.1% DMSO as a vehicle and it did not show any effect.

2.2. Cell culture

RIN-14B, a rat pancreatic islet cell line, was purchased from DS Pharma Biomedical (Osaka, Japan). The cell line was cultured in RPMI 1640 medium (Wako Pure Chemicals) in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. The culture medium was supplemented with 10% heat-inactivated fetal bovine serum (Life Technologies Japan, Tokyo, Japan), 100 μg/ml streptomycin (Meiji Seika Pharma, Tokyo, Japan), and 100 U/ml penicillin (Meiji Seika Pharma).

2.3. Measurement of intracellular Ca^{2+} concentrations

The intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in the cells were measured with the fluorescent Ca^{2+} indicator fura-2 by dual excitation using a fluorescent imaging system controlling illumination and acquisition (Aqua Cosmos; Hamamatsu Photonics, Hamamatsu, Japan), as described previously (Ohta et al., 2008). To load fura-2, cells were incubated for 30 min at 37 °C with 10 μM fura-2 AM (Life Technologies Japan) in HEPES-buffered solution. A coverslip with fura-2-loaded cells was placed in an experimental chamber mounted on the stage of an inverted microscope (IX71; Olympus, Tokyo, Japan) equipped with an image acquisition and analysis system. Cells were illuminated every 5 s with lights at 340 and 380 nm and the fluorescence signals at 500 nm were detected. Emitted fluorescence was projected onto a charge coupled-device camera (ORCA-ER; Hamamatsu Photonics), and the ratios of fluorescent signals (F340/F380) for $[Ca^{2+}]_i$ were stored on the hard disk of a computer (Endeavor Pro 2500; Seiko Epson Co., Nagano, Japan).

2.4. Whole-cell current recording

RIN–14B cells cultured on coverslips were mounted in an experimental chamber and superfused with HEPES-buffered solution as for Ca^{2+} imaging experiments. The pipette solution contained (in mM) 140 CsCl, 10 HEPES, 5 EGTA, 2 MgATP, pH 7.2 with CsOH. The resistance of patch electrodes ranged from 4 to 5 MΩ. The whole-cell currents were sampled at 5 kHz and filtered at 1 kHz using a patch-clamp amplifier (Axopatch 200B; Molecular Devices, Sunnyvale, CA) in conjunction with an A/D converter (Digidata 1322A; Molecular Devices). Membrane potential was clamped at −60 mV and voltage ramp pulses from −100 mV to +100 mV for 100 ms were applied every 5 s.

2.5. 5-Hydroxytrytamine release

RIN–14B cells (2.5×10^5) were seeded in 24-well plates and cultured for 72 h. The medium was removed, and the cells were washed and preincubated in HEPES-buffered solution with or without a blocker at 37 °C for 20 min. Then the cells were washed and treated with or without various stimuli in HEPES-buffered solution containing 2 μM fluoxetine at pH 7.4. Incubation was performed at 37 °C for 20 min. Sample solution was collected and centrifuged. The supernatant was collected and prepared as supernatant assay solution containing 0.4 N perchloric acid (PCA) (Sup sol.). The cells remaining on the plates were extracted with 0.4 N PCA and prepared as a cell assay solution (Cell sol.). These preparations were performed on ice. Measurement of 5-HT was carried out using an HPLC system (HTEC-500; Eicom Co., Kyoto, Japan) equipped with an electrochemical detection system. The samples (10 μl) were injected into the HPLC system. The flow rate was 0.5 ml/min, and the electrodetection was performed at 0.75 V. In the mobile phase, we used 0.1 M acetate-citrate buffer containing 17% methanol, 190 mg/l sodium 1-octanesulfonate, and 5 mg/l EDTA-2Na. The 5-HT secretion ratio of the concentration in Sup sol. to total contents (Cell sol. + Sup sol.) was calculated.

2.6. Cytotoxicity assay with formazan production

The cytotoxicity was assayed by Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) based on a water-soluble tetrazolium salt (WST-8), that produces a water-soluble formazan dye by the cellular dehydrogenase of living cells. To determine the cell viability, the absorbance at 450 nm (A_{450}) was measured by a microplate absorbance reader (Tecan Japan, Kawasaki, Japan).

2.7. Statistical analysis

Data are presented as mean ± SEM. For multiple comparisons, one-way ANOVA was used followed by Dunnett's test. $P < 0.05$ was considered significant.

3. Results

3.1. Methylglyoxal evokes $[Ca^{2+}]_i$ increases, inward currents and 5-HT secretion in RIN–14B cells

In fura-2-loaded RIN–14B cells, MG was applied with different concentrations. Averaged $[Ca^{2+}]_i$ responses to MG at three concentrations in different cells are shown in Fig. 1A. MG (1 mM) gradually elicited $[Ca^{2+}]_i$ increases in RIN–14B cells and the time-to-peak of the response was faster at higher concentration of MG.