Enhanced histamine production through the induction of histidine decarboxylase expression by phorbol ester in Jurkat cells

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Abstract. Histamine (HA), a mediator of inflammation, type I allergic responses and neurotransmission, is synthesized from L-histidine, the reaction of which is catalyzed by histidine decarboxylase (HDC). HDC has been reported to be induced by various stimuli, not only in mast cells and basophils, but also in T lymphocytes and macrophages. Although its mRNA has been shown to be increased in Jurkat cells when treated with phorbol 12-myristate 13-acetate (TPA), little is known concerning the induced production of HA by HDC. The present study quantified the trace amounts of intracellular HA using ultra-high liquid chromatography in combination with the 6-aminoquinoline carbamate-derivatization technique. To test whether the cellular level of HA is elevated by the induction of HDC in Jurkat cells treated with TPA, the peak corresponding to authentic HA in the cell lysate was fractioned and its molecular weight determined by matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight mass spectrometry. The results of this study show that the HA level is increased by the induction of HDC expression by TPA in Jurkat cells. Therefore, this method is useful in elucidating the physiological significance of HA production.

Introduction

Histamine (HA) is involved in a variety of physiological processes, including inflammation, allergic responses, neurotransmission and gastric acid secretion (1,2). HA is synthesized by a pyridoxal phosphate-dependent enzyme, histidine decarboxylase (HDC, EC 4.1.1.22), with L-histidine as a substrate. The genetic expression of HDC has been widely detected throughout the human body (3,4). HDC is reportedly induced by various stimuli, not only in mast cells and basophils, but also in T lymphocytes and macrophages (5,6). In particular, this non-mast cell- and/or non-basophil-derived HA has been shown to be involved in the regulation of angiogenesis, inflammatory granulation and cell proliferation (6,7). It has been demonstrated that cultured T lymphocytes overexpressed the HDC gene in response to concanavalin A or various cytokines and secreted HA into a culture medium (5,8).

In Jurkat cells, a model human cell line for T cells used in numerous studies, HDC gene transcription was markedly enhanced by treatments with phorbol 12-myristate 13-acetate (TPA) or phytohemagglutinin (PHA) (9). However, no studies assessing HA production in TPA-stimulated Jurkat cells are currently available. In the current study, a rapid and high-sensitivity derivatization technique was introduced, used with 6-aminoquinoline carbamic acid (AQC) as a fluorescent reagent (10,11), for detecting the trace amounts of intracellular HA. The cellular level of HA in Jurkat cells treated with TPA was then determined by developing an accurate and reliable method quantified by ultra-high performance liquid chromatography (UPLC) in combination with matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight tandem mass spectrometry (MALDI-QIT-TOF/MS) for the molecular identification of HA.

Materials and methods

Chemicals and reagents. HA, 3-methylhistamine (3-methyl-HA) dihydrochloride, (R)(−)-α-methyl-HA dihydrochloride and TPA were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol and acetonitrile were obtained from Wako (Tokyo, Japan). An AccQ-Tag Ultra-Fluor™ derivatization kit (borate buffer and reagent) was purchased from Waters (Milford, MA, USA). Bradykinin fragment 1-7, the calibration standard for MALDI-QIT-TOF/MS, was purchased from Sigma-Aldrich. 2,5-Dihydroxybenzoic acid (DHBA) matrix of MALDI-MS grade was purchased from Shimadzu GLC (Kyoto, Japan).

Cell culture. Jurkat cells, a human leukemic T-cell line, were maintained in RPMI 1640 complete medium (Wako) with 10% heat-inactivated fetal bovine serum (FBS, Invitrogen,
Carlsbad, CA, USA), 2% (w/v) L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin (Invitrogen). HeLa S3 cells, a human epithelial carcinoma, were maintained in RPMI 1640 supplemented with 5% FBS. The two cell lines were cultured in a humidified atmosphere containing 5% CO₂ at 37°C.

**RNA preparation and RT-PCR.** Total RNA was extracted from cultured Jurkat cells (5x10⁶ cells/dish) and HeLa S3 cells (3x10⁶ cells/dish) treated with TPA (10 ng/ml) or dimethyl sulfoxide (DMSO) for 16 h. The synthesis of cDNA and RT-qPCR was performed as previously described (12). The primers used for amplification were hdc forward, 5'-CAAGCACTAGTCAAGATGG-3'; reverse, 5'-GACGAGAGGAGGACAG-3'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward, 5'-GTCATGATGGCATGGACTGTGGT-3'; GAPDH reverse, 5'-TGAACAGGAAGGAGGACAG-3'.

**Sample preparation and derivatization for chromatographic analysis.** Jurkat cells were plated at 1.25x10⁵ cells/well and harvested following stimulation with DMSO or TPA (10 ng/ml) for 16 h. Cells were homogenized in 0.6-ml Milli-Q water using an ultrasonic cell disruptor on ice. Cell debris and insoluble matter were removed by centrifugation at 14,000 rpm for 10 min at 4°C. The protein concentrations of the lysates were determined by Bio-Rad Protein Assay reagent (Bio-Rad, CA, USA), 2% (w/v) L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin (Invitrogen). HeLa S3 cells, a human epithelial carcinoma, were maintained in RPMI 1640 supplemented with 5% FBS. The two cell lines were cultured in a humidified atmosphere containing 5% CO₂ at 37°C.

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**MALDI-QIT-TOF/MS.** DHBA matrix (5 mg) was dissolved in 0.5 ml of 16% acetonitrile and 0.06% trifluoroacetic acid. Matrix solution (0.5 μl) and 0.5 μl of sample dissolved in Milli-Q water were deposited on a MALDI plate and left to dry at room temperature to prepare sample spots. External calibrations were achieved using the standard reagents of DHBA matrix (monoisotopic mass of [(M+H)⁺ = 155.03] and Bradykinin fragment 1-7 (monoisotopic mass of [(M+H)⁺ = 757.4]). MS experiments with MALDI-QIT-TOF/MS (n=1, 2) were performed using argon as the collision gas on an AXIMA Resonance mass spectrometer (Shimadzu-Biotech, Kyoto, Japan) equipped with a nitrogen laser (337 nm), with the collision-induced dissociation (CID) control value set at 230.

**Standard curves.** Calibration standards for HA (0, 0.25, 0.5, 1.0, 2.0 and 4.0 nmol) were prepared from the stock solution (25 μg/ml) diluted in Milli-Q water and stored at -20°C. For the preparation of the calibration curves, standards for HA and internal standards (2 nmol of α-methylHA) were added to cell lysates and subjected to extraction and chromatography as described above. Calibration curves were calculated by plotting the ratio of the peak area of analytes to the area of the internal standard (α-methylHA) vs. analyte quantity.
Results

Induction of HDC mRNA in Jurkat cells. To confirm a previous result (9), total RNA was extracted from Jurkat and HeLa S3 cells to be used as a negative control and the level of hdc mRNA was examined by an RT-PCR experiment using gene-specific primers for human hdc. As shown in Fig. 1A, the level of hdc mRNA was significantly increased when stimulated with TPA in Jurkat cells. Conversely, it was not detected in HeLa S3 cells with or without TPA (Fig. 1B).

Development of methods. HA, 3-methylHA and α-methylHA were fluorescently derivatized with AQC as described in Materials and methods. Typical UPLC (equipped with ODS column) chromatograms were obtained following the injection of standard samples containing AQC-derivatized standard HA-derivatives (Fig. 2A). Retention times of AQC-HA (peak I), AQC-3-methylHA (peak II) and AQC-α-methylHA (peak III) were 35.9, 48.6 and 52.3 min, respectively. Representative chromatograms of samples from non- and TPA-stimulated Jurkat cells are shown in Fig. 2B and C, respectively.

MS and MS/MS. To assess the identity of the peak derived from Jurkat cells, the peak was fractioned with the retention time corresponding to that of the AQC-derivatized standard HA and mass analysis with MALDI-QIT-TOF/MS was carried out. Representative MS and MS/MS spectra of the putative AQC-HA ([M+H]+ = 281.4) derived from Jurkat cells were observed (Fig. 3A). Following CID, the parental ion produced the fragment ion 170.94 (the eliminated AQC-group). Specific daughter transition for AQC-HA≥HA was revealed to be of 112.23 molecular mass (m/z)

Calibration and quantitative analyses. A calibration curve was calculated using the peak area vs. concentrations of the standard analyte. The linearity was verified over the assay range (0-4.0 nmol on-column). The typical linear regression equation from the standard curve for HA was y=5.013x10^5x where y was the peak area ratio of analyte to internal standard and x the analyte amount (nmol). The correlation coefficient for the standard in cell lysates was 0.999 (Fig. 4A). Under this condition, the intracellular HA was measured and the increase in its content from 4.28 (untreated) to 786.97 pmol/mg protein (treated with TPA) was identified (Fig. 4B) through the induction of the HDC gene expression (Fig. 1A).
Discussion

The transcriptional activation of HDC has been proven to be controlled by the gastrin-responsive element located in the 5’-region of its genome (14). Moreover, treatment with TPA and/or PHA enhances this promoter activity in Jurkat cells (9). However, little is known about the induced production of HA in cultured cells. The present study demonstrates that the production of HA was upregulated in association with HDC induction stimulated by TPA in Jurkat cells.

Quantitative analyses of HA by HPLC have been previously reported using fluorescence derivatization with dansyl chloride (15) or o-phenaldehydes (13). However, these conventional methods are too time-consuming to complete the reaction (15) or require the acidification of the reaction mixture to stabilize the fluorophore (13). The current study adopted a sensitive and accurate method for quantifying HA by UPLC combined with an AQC-labeling technique (16-18), which is a more rapid and stable method than those used previously; the derivatization is completed in under 1 min and AQC-derivatives are immediately applied to the UPLC system. By using the elution program described in Materials and methods, standard AQC-HA (peak I) was observed as the single sharp peak and AQC-α-methylHA (peak III) was also detected to allow accurate integration for quantification as an internal standard (Fig. 2A).

A significantly increased peak was identified at an identical position to the AQC-standard HA in the lysates of the Jurkat cells treated under conditions described in Fig. 1A (Fig. 2C), with this peak being collected for MS analysis. Since the MS ≥ MS/MS sequential analysis with MALDI-QIT-TOF/MS revealed that the CID patterns of the UPLC-fractioned peak (Fig. 3A) were identical to those of the AQC-derivatized standard HA (Fig. 3B), we concluded that the substance increased by the TPA-stimulation in Jurkat cells was HA. Since the linear regression analysis demonstrated significant linearity of the calibration curve from 0 to 4.0 nmol (Fig. 4A), the intracellular HA was measured with this calibration curve and revealed that the amount of HA had increased to approximately 180-fold by TPA, compared with the non-stimulated cells (Fig. 4B).

HA is metabolized in two ways: one is the oxidative deamination by diamine oxidase (DAO, EC 1.4.3.22) and the other is imidazole ring-methylation by histamine-N-methyltransferase (HNMT, EC 2.1.1.8) that converts HA to 3-methylHA only in the cytoplasm (19-21). No peaks were detected in Fig. 2 corresponding to the standard 3-methylHA derivatized with AQC in TPA-stimulated cells and/or control cells, although the HNMT gene was expressed in the Jurkat cells treated with or without TPA (data not shown). The reason for 3-methylHA not being detected in the HDC-induced cells may be as follows: i) low contents of 3-methylHA below measurable limits; ii) ineffective translational rates of HNMT; iii) inactivation or degradation of HNMT; or iv) rapid secretion of 3-methylHA into culture media.

Results of a previous study have demonstrated that the overproduced HA in T lymphocytes stimulated by concanavalin-A was secreted to the extracellular space (8). However, the mechanisms of secretion and/or metabolism remain to be determined. Therefore, additional investigation of the culture medium is required for the estimation of the total cellular production of HA in T lymphocytes.

In conclusion, the method described in this study is useful for elucidating the physiological significance of HA production, not only in T lymphocytes, but also in other cell types.

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