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Review

Assay and biological relevance of endogenous histamine and its metabolites: application of microseparation techniques

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Abstract

This review provides an overview of the assay methods used to determine the presence of endogenous histamine (HA) including its metabolites, and also discusses their biological significance. Firstly, this review briefly summarizes the biological significance of HA and its biological pathways. Next, the assay methods with microseparation techniques, such as gas-chromatography (GC), liquid-chromatography (LC), capillary electrophoresis (CE) and capillary electrochromatography (CEC) are looked at from a developmental viewpoint. Finally, the use of these methods, including flow cytometry techniques, for the determination of HA and its metabolites in biological samples, such as blood, urine, brain and cells, is described. The merits and demerits associated with each of these various methods are also discussed, along with their applications.

Keywords: Reviews; Histamine

Contents

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1. Introduction

Histamine (HA) research has a long history starting at the beginning of the 20th century. About one century has passed, and many results have been accumulated by researchers who have attempted to clarify just what biological role HA plays in living systems, with the result that the complete picture of the various roles played by HA will likely be explained in the near future. Actually, the remarkable advances in HA research has been impelled and accelerated by the developments of effective biological and/or chemical assay methods. However, recent advances in our understanding of the role of the biologically active HA in animals have brought to light many unsolved problems serving to emphasize the need for rapid, accurate, and specific methods for HA measurement. In this review, we broadly summarize the assay methods of HA and its related compounds using microseparation techniques, from outmoded classical methods to the latest techniques, with regard to the biological relevance of HA.

2. Biology

2.1. Biological significance

HA is an important chemical mediator, not only in peripheral tissues as in type I allergic diseases and digestive ulcers, but also in the central nervous systems (CNS). Recently, it has become possible to investigate ligand receptor interaction at the molecular level using molecular biological techniques, and, by doing so, we have come to understand that HA exerts multiple biological actions through one of three receptor subtypes (H1, H2, and H3).

Allergies are abnormal responses of the body to usually harmless things as well as to known allergens that one encounters in daily life. When the first infection with an allergen occurs in the mammalian body, a specific immunoglobulin E (IgE) antibody is produced against the particular allergen. The IgE binds to an IgE receptor that is located on the surface of mast cells or other types of cells. When, after the second infection by the same allergen occurs, the allergen binds and cross-links two or more of the IgE antibodies attached to IgE receptor located on the cell surfaces of mast cells or basophils, HA is released in quantity. Released HA causes seasonal allergic rhinitis (SAR) and asthma by binding to widely distributed H1 receptors. The H1 receptor antagonists (antihistamines) are an important class of medications used for the relief of common symptoms associated with hyperhistaminic conditions occurring in both children and adults [1].

HA is also secreted from enterochromaffin-like (ECL) cells in response to circulating gastrin, thereby stimulating the H2 receptors, whose main function is to trigger the secretion of gastric acid. The H2 antagonist is one of the most useful chemotherapeutic drugs used in the treatment of gastric ulcer [2].

HA acts on target cells in the mammalian brain via stimulation of two classes of receptor (H1 and H2) as second messengers. It is well established that several neurotransmitters affect neuronal activity in the central nervous system through stimulation not only of postsynaptic receptors, but also of presynaptically-located receptors, which often display distinct pharmacological specificity and by which they may control their own release. This type of receptor is usually referred to as an ‘‘autoreceptor’’. Recently, HA was shown to inhibit its own release from depolarized slices of rat cerebral cortex. This phenomenon was found to be mediated by a novel class of HA receptor as the autoreceptor, the H3-receptor, which was found to be pharmacologically distinct from the H1- and H2-receptors [3]. Up to now, there have been no indications whether this third class of histamine receptor is present in any tissue other than the brain.

Recently, several groups have suggested the existence of H4 receptors by identified cDNA encoding. All of the groups have initially found a clue for the H4 receptor-nucleotides sequence in the draft of the human genomic DNA database. According to their reports, the H4 receptor binds to HA with high affinity, which results in the down-regulation of intracellular cAMP. The H4 receptor is localized in the peripheral blood leukocytes, spleen, thymus, small intestine, colon, bone marrow and so on. The tissue distribution of the H4 receptor and known physiological function of histamine tempts us to speculate about its function as an immune modulator. Although there needs to be much additional work on

the characterization of the H4 receptor, the discovery of this receptor subtype unveils a new phase for determining the physiological role of HA [4–6]. Currently, much attention is being paid to the various histamine receptors and current research is focused on the elucidation of the multiple biological functions of HA and it metabolites in the mammalian body, and the therapeutic potential of these receptor ligands.

In the near future, the whole picture of the role of HA in mammalian body will be clarified by both biological and analytical methods.

2.2. Biological pathways

HA is biologically synthesized from histidine by histidine decarboxylase (HDC) in mast cells, basophils and many cells including normal and malignant lymphocytes, and is stored within granules in these cells. HA which has been released into blood or body tissue is metabolized via three enzymatic pathways as shown in Fig. 1. In the first pathway, HA is deaminated by diamine oxidase (DAO or histaminase) to form imidazoleacetic acid (IAA) vie 4-imidazolecarboxyaldehyde. In the second pathway, HA is methylated to form 1-methylhistamine or N^{τ}-methylhistamine (MHA) by the enzyme, histamine-*N*-methyltransferase. MHA is subsequently deaminated by monoamine oxidase to form 1-methylimidazoleacetic acid or N^{τ}-methylimidazoleacetic acid (MIAA) vie 1-methyl-4-imidazolecarboxyaldehyde. In the last pathway, HA is acetylated to form 4-(β-acetylaminoethyl)imidazole by the enzyme acetylase that has been identified only from enterobacteria, but not within the mammalian body.

On these biological pathways, it is known that HDC activity increases with decreasing Mg levels. In contrast, DAO activity decreases with decreasing Mg levels. Actually, the HA levels in the urine of rats that were fed a Mg-deficient diet were higher than those of rats whose diet was rich in Mg [7]. The influences on HA and its metabolite levels by other factors such as foods [8], vitamins [9,10], drugs [11], age [12], sex [13] etc. were also pointed out.

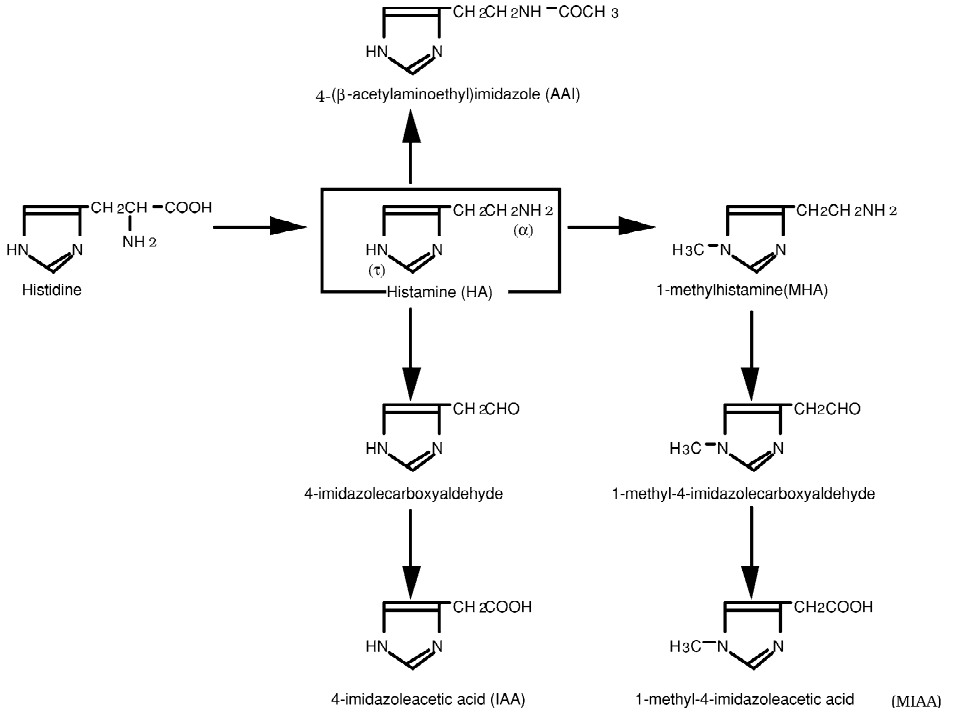

Fig. 1. Enzymatic pathways of HA and its metabolites in mammals.

Therefore, such external factors are taken into special consideration when the assays of HA and its metabolites are carried out.

3. Chromatography

To measure HA and related compounds in biological samples, very sensitive analytical techniques are required. Radioenzymatic assay (REA) is one useful technique for this purpose. REA is the name used to describe an analytical technique that uses an enzyme and radioisotope-labeled co-substrate to quantify another co-substrate of the enzyme by reproducibly converting it to radioisotope-labeled product. Such an assay method using histamine *N*-methyltransferase was developed for the quantification of HA [14]. Radioimmunoassay (RIA) or enzymeimmunoassay (EIA) are also important analytical tools, in addition to REA. These methods are based on the specific immunogenic coagulation which occurs between antigen and antibody. The key point to the development of these techniques is to first produce either monoclonal or polyclonal antibodies to HA or its analogs. However, it is relatively hard to prepare these antibodies so that they possess both the sensitivity and specificity required for clinical purposes, because HA and its analogs are small molecules and therefore haptens with antigenic, but not immunogenic potency. Generally, the generation of specific antibodies directed towards haptens is difficult, unless these are modified into effective immunogens [15].

Along these lines, chromatographic microseparation techniques, such as gas chromatography (GC), high-performance liquid chromatography (LC), capillary electrophoresis (CE) and capillary electrochromatography (CEC) have appeared as alternative tools, because these techniques offer both simultaneous and high-sensitivity detection of HA and its analogs in biological samples. These techniques are now routinely applied to achieve separation of HA and it metabolites adequate for use in clinical laboratories.

3.1. Gas chromatography (GC)

GC assay methods can be applied to HA and its metabolites directly, but some problems associated with separation occur with this method. The main problem is adsorption, which results in peak tailing and a memory effect. To overcome both of these effects, acyl groups are introduced into the amines by means of suitable derivatization reagent or, alternatively, a deactivated GC system is generally employed. Derivatization of HA and its metabolites improves elution and resolution with considerable enhancement overall in the chromatographic properties.

The usual method employed to derivatize the molecules is to acylate the primary amino group at the N^{α} position and/or secondary amino group at the N^{τ} position in the imidazol ring of HA and its metabolites. At the N^{α} position of the molecule, several fluorogenated derivative reagents including, pentafluoropropionyl anhydride (PFPAn) [16,32], 2,6-dinitro-4-trifluoromethylbenzensulfonic acid (DNTSA) [19], heptafluorobutyl anhydride (HFBAn) [20,21,24,25,27], 3,5-bistrifluoromethylbenzoyl chloride (bis-TFMBO-Cl) [29], trifluoroacetyl anhydride (TFAAn) [30] and pentafluorobenzyl bromide (PFB-Br) [30] are usually employed because the high-fluorine content derivatives ensure adequate volatility under normal GC conditions. For derivatizing the secondary amine at the N^{τ} position, acetic anhydride (AAn) and methyl chloroformate were used to introduce an acetyl and a methoxycarbonyl group, respectively.

For HA, a double derivatization method at both N^{α} and N^{τ} positions is sometimes employed. MIAA, a major metabolite of HA, is converted into its ester derivative with methanol/HCl [17], ethanol/HCl [18], 2-propanol/HCl [23], boron trifluoride-butanol [26], 3,5-bistrifluoromethylbenzoyl chloride [28] and pentafluorobenzyl bromide [30].

HA and its metabolite derivatives were subsequently separated with a glass tube silanized with hexamethyldisilazane [17,19,26] and packed with particles coated with either alkyl or phenyl polysiloxane, or were separated with a fused-silica capillary tube the inner glass wall of which was usually coated with polyethyleneglycol (Carbowax) [23] or polysiloxane [22,25,28–30,32,33]. Various detection devices are employed for the GC systems, including hydrogen flame ionization detector (FID) [17,18], electron-capture detector (ECD) [19], nitrogen–phosphorus detector (NPD) [23,24,27] and mass-spectrometer (MS). The NPD provides additional

selectivity in measuring primarily nitrogen-containing molecules and has been found particularly useful for the quantitation of imidazoles. Alternatively, greater sensitivity was achieved by ECD when fluorogenated derivatives were employed, but was achieved at the cost of selectivity. This is the other reason why fluorogenated derivatizing reagents are generally employed to convert HA and its metabolites to volatile compounds for GC analyses.

MS is the best detection device for GC determination of these amines with high selectivity and high sensitivity. This device generally connects to a GC system on-line, and, hence, is given a hyphenated label, e.g. GC–MS. With this method, HA derivatives, separated from the GC column, ionized by electron impact (EI) [20,21,30], chemical ionization (CI) with ammonia [25,28,29,31,33], or isobutane [26], and the ions thus formed are subsequently monitored by SIM (selective ion monitoring mode). In order to determine the concentration of the desired constituents in a sample with this system, a stable isotope dilution method is usually employed. This method is based on calculating the molecular ion abundance ratios of the desired compound to the synthetic internal standard (I.S.) which was obtained by chemical replacement of H or N atoms in the desired compound with stable isotopes such as ^{2}H or ^{15}N. After injection of the sample containing the I.S. to the GC–MS, the desired compound and the I.S. co-migrate on the column, and give a fused peak on the chromatograph. Then the fused peak is monitored with SIM to give both the molecular ion abundance of the desired compound and the I.S. This method has both good accuracy and reliability for analyses of HA and related compounds in biological fluids.

Several applications of analyses of HA and its metabolites in biological samples with GC are listed in Table 1.

3.2. Liquid chromatography (LC)

The LC system is another very attractive method for the simultaneous determination of HA and its metabolites in biological samples. Generally, one of the advantages of LC is that it can be applied even to samples which are unstable at high temperatures, because there is no need to heat the samples in order to convert them to gaseous form before injection into the separation column. Most analytes, including non-volatile molecules, can be directly separated on various columns whether the analytes are derivatized or not. To separate HA and its analogues, cation-exchange [34,36,43,47], cyanopropyl [54,55] or reversed-phase column [35,38–42,44–46,49–53,56] using ion-pairing reagents are all commonly employed in LC separation assay.

On the other hand, one of the disadvantages with LC is that there is no universal detection device equivalent to MS, as in the case of GC. An interface for introducing the analytes to an ionization chamber in MS equipment after LC separation has also been developed, and applied to biological samples. However, the reported assay method for HA with LC equipped with MS as a detector is very limited because it is very hard to remove or separate the analytes from the non-volatile constituents in a mobile phase for LC (unlike situations with GC–MS). For further discussion of this, see Bolygo et al. [57], who reported on the determination of HA in tomatoes by GC–MS. Several detection devices, such as ultra-violet (UV) absorption [42], fluorescence [34–41,44,46,48–51,53–56], electrochemical detectors [40,43,45,52] and chemiluminescence [57] have been developed and applied as high sensitivity and selectivity detection devices to the amines discussed here. UV detection is the tool in general use for this purpose, because HA has a characteristic UV spectra and a high extinction coefficient of around 210 nm wavelength. With UV, Hui and Taylor [42] reported that the detection limit of native HA and MHA in urine might be reached at around the μmol/ml level after separation with ion-paired reversed-phased LC. However, this detection sensitivity is not enough to determine HA in some cases. In such cases, pre-, post- and on-column derivatization methods are generally carried out by performing chemical reaction of the amino group (or N^{α} position) of HA with *o*-phthalaldehyde (OPA) [34–41,45,46,49–56] or fluorescamine [44] as the derivatizing reagents in order to obtain high detection sensitivities using a fluorescence detector. In addition, a fully automatic system is much easier to set up with a post- or on-column derivatization platform than with a pre-column derivatization platform, because the post-column and on-column derivatizations are carried out within an additional reaction device connected to the separation column and in the separation column itself, respectively,

Table 1
GC assay methods in biological samples

Analyte/sample	Derivatization	Column	Detection	Reference
MIAA/urine	Methanol/HCl	Silanized glass column coated with 1% polyvinylpyrrolidone (10% EGA on Gas-Chrom P)	FID	[17]
MIAA/urine	Ethanol/HCl	Glass column coated with 1% polyvinylpyrrolidone (6% EGA on Diatoport S)	FID	[18]
HA, MHA/urine	DNTSA	Silanized glass columns (3% dimethylsiloxane or 3% alkylpolysiloxane on Supelcoport)	ECD	[19]
HA, MHA/plasma, urine	HFBAn + ethyl chloroformate	Glass column (5% dimethylsiloxane on Supelcoport)	MS(EI)	[20,21]
MHIS/urine	bis-TFMBO-Cl	Capillary column (coated with 5% phenyl–95% methylpolysiloxane)	MS	[22]
MIAA/urine	2-Propanol/HCl	Capillary column (coated with Carbowax)	NPD	[23]
MHA/urine	HFB-Br	Capillary column (coated with 100% dimethylpolysiloxane)	NPD	[24]
HA/plasma	HFB-Br	Capillary column (coated with 100% dimethylpolysiloxane)	MS(CI)	[25]
MIAA/blood, plasma, urine	boron trifluoride /butanol	Silanized glass column (1% Poly A-135 + 2% Silar 5 CP on Gas-Chrom Q)	MS(CI)	[26]
HA, MHA/urine	HFB-Br + AAn	Glass column (3% OV-275 on Chromosorb)	NPD	[27]
MIAA/urine	bis-TFMBO-Cl	Capillary column (coated with 5% phenyl–95% methylpolysiloxane)	MS(CI)	[28]
MHA/urine	bis-TFMBO-Cl	Capillary column (coated with 5% phenyl–95% methylpolysiloxane)	MS(ECNICI)	[29]
MHA, MIAA/urine	TFAAN/HFBAn	Capillary column (coated with 100% dimethylpolysiloxane)	MS(EI)	[30]
IAA/urine	Boron trifluoride-butanol	Glass column (1% poly A-135 + 2% silar 5CP on Gas-Chrom Q)	MS(CI)	[31]
MHA/urine	PEPAn	Capillary column (coated with 100% dimethylpolysiloxane)	MS(EI)	[32]
MA, MHA/plasma	PFB-Br, HFBAn	Capillary column (coated with 100% dimethylpolysiloxane)	MS(CI)	[33]

Abbreviations are listed in the text.

where the analyte is reacted with the given reagent automatically. However, the post- and on-column methods result in broader peaks of its derivatives than the pre-column method, due to their mixing of the analyte and reagent in the reaction device. The detection sensitivity of the detection device with fluorescamine and OPA are almost the same at around several pg on-column [44].

OPA is most commonly used as the derivatizing reagent for amine, but OPA requires carefully controlled reaction conditions and duration of derivatization, because the derivatization products of OPA have limited stability. Therefore, pre-column derivatization with fluorescamine is more suitable than OPA because fluorescamine derivatives show greater stability. Actually, post-column derivatization platforms using OPA were developed in order to determine HA and its metabolites [34–38,41].

In another approach to enhance the detection sensitivity, a LC-chemiluminescence post-column

method was examined by Alam et al. [57]. They reported using this method for the simultaneous determination of HA and MHA in biological sample tissues with the LC-chemiluminescence post-column method as shown in Fig. 2. HA and MHA were separated on an octadecyl silane (ODS or C_{18}) column and eluted with 0.1 *M* potassium phosphate buffer (pH 6), and subsequently passed through a column packed with TSK-gel (Tosho, Tokyo, Japan) that was covalently immobilized with diamine oxidase (DAO). Chemiluminescence was detected, formed by the reaction of a luminol–ferricyanide mixture in alkaline medium with hydrogen peroxide, which is one of the metabolic products of HA and MHA formed by DAO. The LC with post-column derivatization resulted in good separation of the two amines and gave linear relationships between the concentrations of both amines and their chemiluminescence intensities. The lower limits of chemiluminescence detection of HA and MHA were 5 and 10 pmol, respectively.

Although GC or LC usually requires derivatization, the purposes of the derivatizations are quite different from each other. In the case of GC or GC–MS, the derivatization is carried out in order to convert the compounds to volatile compounds having good chromatographic properties. On the other hand, the aim of derivatization in the case of LC is to enhance detection sensitivity and/or selectivity. Although the combined methods consisted of derivatization, and LC, GC or GC–MS methods have excellent properties regarding separation or detection sensitivity, they are not easy to use in routine analyses. Therefore several approaches have been attempted to overcome this problem. For example, Saito et al. first proposed the idea of on-column methods using a mobile phase containing OPA reagent. During the time when a free analyte is moving on a column in a mobile phase, the free analyte is derivatized with the reagent in the mobile phase, and the analyte is separated and detected automatically. Detection sensitivity in this case was almost equivalent to other labeling methods with OPA, and they reported the detection limit of several nmol for HA or MHA per ml of sample solution. This approach decreased or eliminated the manual sample treatments or the setting up of additional equipment that were needed by authentic pre- or post-column methods.

For another approach, the electrochemical oxidation method as an alternative detection platform was developed for the detection of native HA and its metabolites at high sensitivity. The greatest merit of using electrochemical detection devices for LC analysis is that derivatization is not essential to obtain high detection sensitivity. Houdi et al. [47] developed a simple and sensitive method for simultaneous determination of native HA and its metabolites

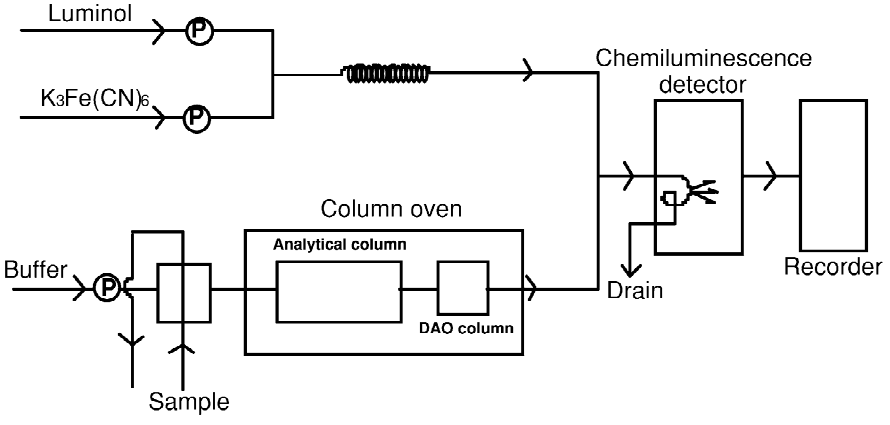

Fig. 2. Flow diagram of the HPLC-chemiluminescence system coupled with immobilized DAO. Analytical column: TSK-gel ODS 80Ts (150 mm×4.6 mm I.D.). DAO column: DAO conjugated with TSK-gel Tresy-5PW (30 mm×4.6 mm I.D.). Reaction solutions consisted of 0.75 m*M* luminol in 0.1 *M* potassium phosphate buffer (pH 6.4) and 12.5 m*M* potassium ferricyanide in 0.35 *M* potassium hydroxide. Elution buffer: 0.1 *M* potassium phosphate buffer (pH 6.4). Reproduced from Ref. [57].

in biological fluids by LC coupled with electrochemical detection. The method involved free HA and MHA, which were separated on an ODS column with 0.1 *M* phosphate buffer–methanol (75:25) containing 3.2 ml of NaOH and sodium dodecyl sulfate (SDS) per 1000 ml as a mobile phase, and subsequent detection occurred at the pmol level.

The application of LC assay methods for determination of HA and its metabolites is shown in Table 2.

3.3. Capillary electrophoresis (CE) and capillary electrochromatography (CEC)

Recently, CE or CEC have been developed to allow analysis of very small volumes of the sample with higher speed and greater specificity in comparison with classical chromatographic methods.

After the first trial uses of CE by Jorgenson and Lukacs [58] in the early 1980s, Manz et al. [59] succeeded in the separation of amino acids on a glass chip in 1992 using CE. Subsequently, there have been major efforts to perfect the fabrication and miniaturization of these analytical systems on a chip in order to carry out high-throughput analyses, with reduced analysis time and human error. Given this background, and because of its high resolution and the possibility of employing ''fabrication on a chip'' technology, CE has come to be a very attractive technique. However, due to the occurrence of several difficulties with CE, there are very few reports on its application for HA analyses. One problem is the inherent decrease in sensitivity of detection due to the shortened pathlength of light. To overcome this problem, methods employing laser-induced fluorimetry (LIF), electrochemical detection and MS have been attempted. MS is employed more appropriately as a detection device for CE than for LC. Girardeau and Gonnord developed a method of detecting amino acids in blood using CE coupled with MS as the detection device [60]. Indirect detection techniques are universally employed for high-sensitivity detection with CE. The method is based on the idea that native analytes can be

Table 2
LC assay methods in biological samples

Analyte/sample	Column	Detection	Reference
HA/plasma, blood	Cation-exchange	Flu (post-column OPA)	[34]
HA, MHA/plasma, urine	Ion-paired C_{18}	Flu (post-column OPA)	[35]
HA, MHA/plasma, blood	Cation-exchange	Flu (post-column OPA)	[36]
HA/plasma	Sulfonated polystyrene column	Flu (post-column OPA)	[37]
HA/tongue	C_{18}	Flu and Elec (post-column OPA)	[38]
HA/tissue	C_{18}	Flu (pre-column OPA)	[39]
HA/blood	C_{18}	Elec (pre-column OPA/2ME)	[40]
HA/plasma, urine, tissue	C_{18}	Flu (post-column OPA)	[41]
HA, MHA, MIAA/urine	Ion-paired C_{18}	Colo (212 nm)	[42]
HA, MHA/blood	Cation-exchange	Elec (pre-column Bolton-Hunter reagent)	[43]
HA, MHA/organs	C_{18}	Flu (pre-column fluorescamine)	[44]
HA, MHA/plasma, urine	C_{18}	Elec (pre-column OPA/2ME)	[45]
HA/blood, organs	Ion-paired C_{18}	Flu (pre-column OPA)	[46]
HA, MHA/plasma, urine	Cation-exchange	Elec	[47]
HA, MHA/plasma, urine	C_{18}	Flu (on-column OPA)	[48,49]
HA/P, blood	Ion-paired C_8	Flu (pre-column OPA)	[50]
HA/sputum	C_{18}	Flu (pre-column OPA/2ME)	[51]
HA/plasma	C_{18}	Elec (pre-column OPA/2ME)	[52]
HA, MHA/blood, organs	C_{18}	Flu (pre-column OPA/2ME)	[53]
HA/blood	Cyanopropyl	Flu (pre-column OPA)	[54]
HA/blood	Cyanopropyl	Flu (pre-column OPA/2ME)	[55]
HA/plasma, urine	Ion-paired C_{18}	Flu (pre-column OPA)	[56]

Flu, fluorimetry; Elec, electrochemistry; Colo, colorimetry. Other abbreviations are listed in text.

indirectly detected in the capillary using a run buffer containing a background electrolyte (BGE) in which a chromophore or fluorophore is present. With this ''system on a chip'', determinations of biogenic amines, including HA in food [61] and amino acids in urine [62], were attempted. Recently, Nishiwaki et al. [63] reported a method for determination of urinary HA and MHA using CE with UV detection at 210 nm. After purification of a urine sample with a silica cartridge, HA and related compounds were electrophoresed using a borate buffer (pH 9) containing 100 m*M* SDS as run buffer. With this system, they reported that the HA and MHA levels in urine from a normal female volunteer, and a male bronchial asthma patient, clearly rose after eating or after an asthma attack, respectively.

CEC, a hybrid microseparation technique for LC and CE was first demonstrated by Pretorious et al. [65] in 1974. Due to remarkable advances in the methods of instrumentation for CEC, the number of applications has greatly increased in the last decade [64,66]. Almost all were applications on hydrophobic compound separation, but experiments on hydrophilic compounds, such as biogenic amines or amino acids also appeared. Recently, Oguri et al. [67] demonstrated the separation of several biological amines including HA with on-capillary column derivatization CEC using a run buffer consisting of 5 m*M* OPA/2ME in 10 m*M* borate buffer (pH 10)–acetonitrile (3:7, v/v) with OPA/2ME as a derivatization reagent.

In spite of its high potential as an analytical tool, applications of CE for determination of HA and its metabolites in biological samples have been very limited until now, but the number of applications using these methods will undoubtedly increase over those employing the more classical GC and LC techniques.

4. Endogenous HA and its metabolites in biological samples

4.1. Blood and urine

Assay methods for determination of plasma, serum or urinary HA levels require much higher sensitivity than for its metabolites, because HA released into the blood from mast cells or basophils disappears rapidly as a result of enzymatic degradation which forms stable MHA and other metabolites (Fig. 1), and which are subsequently excreted in urine. Sheinman et al. [68] examined the dose–response to plasma levels of HA and MHA by the intravenous infusion of histamine acid phosphate in six normal volunteers. With increased infusion rate, plasma HA increased, accompanied by a significant, dose-related fall in mean diastolic blood pressure and an increase in pulse rate. All subjects exhibited facial flushing, the threshold plasma HA level for this effect being 1.3 ± 0.15 ng/ml corresponding to an infusion rate of 60 ng/kg per min. In addition, they also suggested in their paper that MHA was unlikely to provide a useful index of HA release into the circulatory system because MHA was seen in only one subject, who exhibited a level of 0.5 ng/ml at the highest infusion rate (180 ng/kg per min). According to other reported data [69], the half-life time of HA in blood after HA labeled with a radio isotope was administered into a mammalian body was measured to be about 1–2 min, and 50, 5–8 and 20% of the doses of HA were excreted in urine as MHA, MIAA and IAA, respectively. The concentrations of HA and its metabolites in total blood, plasma, serum and urine obtained from healthy human adults are shown in Table 3. This suggested that the detection sensitivity for HA and other compounds should be less than the ng/ml level (or pmol/ml) for the study of plasma and urine levels of HA.

The determination of HA metabolites in urine provides a good parameter for measuring HA release. Therefore, the chromatographic methods, GC, LC and CE have been usually employed, because these chromatographic methods can perform simultaneous determinations of HA and its metabolites. Mita et al. [20] described a sensitive method for the simultaneous determination of HA and MHA in human plasma and urine using a GC–MS method. Before GC–MS assay, the flowing steps were required: (1) deproteinization with ethanol, (2) extraction of HA and MHA with butanol, (3) derivatization with heptafluorobutyric anhydride, (4) purification of derivatives by a small-sized column packed with CPG-10 cation-exchanger, and (4) analyzing with GC–MS. With this method, plasma and urinary HA from healthy volunteers were de-

Table 3
Blood, serum, plasma and urine HA and its analogues in healthy adult human [69]

	Whole blood (pmol/ml)	Serum (pmol/ml)	Plasma (pmol/ml)	Urine
HA	100–500	2–25	0.1–1	90–2700 pmol/mg creatinine
MHA	–	–	–	790–2900 pmol/mg creatinine
MIAA	–	–	–	3500–23000 pmol/mg creatinine
IAA	–	–	–	7100±300 pmol/ml

Abbreviations are listed in the text.

termined to be 0.83±0.37 (SD, $n=5$) ng/ml and 34.3±12.9 (SD, $n=4$) ng/ml, respectively. Although plasma MHA in most subjects was below the detection limit, urinary MHA was determined as 157.1±82.0 (SD, $n=4$) ng/ml.

Martens-Lobenhoffer and Neumann [30] developed the determination of the metabolites of MHA and MIAA in human urine by capillary column GC–MS, which happens to be a useful tool for the diagnosis of mastocytosis. Mastocytosis is a disorder characterized by abnormal proliferation of mast cells, which results in an above-normal excretion of HA metabolites in urine. MHA was extracted under basic conditions with chloroform and derivatized with trifluoroacetic anhydride. MIAA was also derivatized with pentafluorobenzylbromide prior to extraction under basic conditions and the derivative was extracted with chloroform. The samples were assayed with GC–MS. The derivatives of MHA and MIAA were separated on an RTX-1 capillary column (15 m length and 0.25 mm I.D.) with helium gas, and ionized at 70 eV prior to monitoring the molecular ions at m/z 95 and 221 for MHA and at m/z 93 and 317 for MIIA. Using this method, MIAA and MHA were found at concentrations of 2.01 and 21.3 μmol/l, respectively, in healthy volunteers' urine samples.

HA assay methods are also useful tools for understanding the relationships between HA and the symptoms of certain diseases. For example, Skoner et al. [70] recently reported on the determination of levels of human urinary HA and its metabolites by GC–MS spectrometry in order to verify whether or not the HA level increases when humans become infected with type-A influenza virus. Healthy adults ($n=15$) were cloistered and inoculated intranasally with type-A influenza virus, and monitored for infection and illness. Daily morning-void urines were collected and assayed for HA and its metabolites by GC–MS. All subjects were infected and developed illness. ANOVA documented a significant effect on urinary levels of total HA ($P<0.02$) on the day the subjects were inoculated, and pairwise (correlation and regression) analysis showed a significant elevation 2 days after inoculation. This result provides the first direct evidence that HA is released in vivo during infection with a virus that causes cold and flu symptoms.

4.2. Brain and other organs

Brain HA is one of the most interesting biogenic amines in brain research for understanding the central histaminergic system, which regulates various physiological functions of the neuroendocrine and cardiovascular systems, e.g. thermoregulation, the circadian rhythm of sleep and wakefulness, and other various behaviors including feeding, drinking, learning, memory, sexual and aggressive behaviours [71]. To determine the levels of HA and its metabolites in biological samples including the brain or other organs using chromatographic techniques, the pretreatments of the sample are the same as for plasma or urinary HA, and are necessary to remove undesired compounds or to concentrate the desired compound either by means of acid extraction or solid-phase extraction. For example, Haaster et al. [44] developed a high sensitivity LC method for the simultaneous determination of both HA and MHA by

pre-column derivatization with fluorescamine. After purification and concentration of the analytes in a extract of a rat brain or other organs with a small column packed with cation-exchange resin, both HA and MHA in the extract were derivatized prior to separation on a reversed-phase ODS column and detected with fluorimetry. According to their results, the average amount of HA was 3.7 ± 3 μg/g wet wt., but MHA was not observed.

Alam et al. [57] reported on a method for the simultaneous determination of HA and MHA in rat brain and peripheral tissues using LC with a post-column derivatization system. After purification of HA and its metabolites in rat brain and peripheral tissues using cation-exchange resin, Amberlite CG50, followed by concentration with lyophilization, HA and MHA levels were measured. The HA levels in the cerebral cortex, forestomach, glandular stomach, and kidney of Wistar rats were found to be 0.30, 58, 396, and 2.4 nmol/g wet wt., respectively. The MHA contents of these tissues were 0.36, 0.40, 0.72, and 3.8 nmol/g wet wt., respectively.

To reduce the number of samples requiring handling, researchers came up with various ideas. For example, Arakawa et al. [37] developed a convenient method for the routine measurement of HA in biological samples that did not require any preliminary purification. This method consists of the direct application of the acid-deproteinized sample to LC on a sulfonated polystyrene column with detection by means of a post-column fluorogenic reaction with OPA; with this method, only 0.1 ml of the sample was necessary for making a determination. The detection limit was found to be 0.1 pmol. Using this method, HA levels for the whole blood and plasma of humans and various animals, or the supernatant of rat peritoneal mast cell incubates and extracts of mouse brain and stomach were also described.

With the on-column CEC method developed by Oguri et al. [67], HA and other biogenic amines were determined in a biological sample without pre-treatment such as solid-phase extraction. When this method was applied to a mixture containing five biogenic amines (HA, serotonin, tyramine, putrescine and cadaverine) and 17 amino acids, the five biogenic amines plus arginine selectively entered into the capillary by means of a voltage injection, and were observed on the electrochromatogram, but none of the amino acids lacking arginine were detected.

In another determination of very small amounts of HA in brain tissue, Mochizuki et al. [72] demonstrated the determination of endogenous HA in rat brain using an in vivo microdialysis technique coupled with LC. The in vivo microdialysis technique is a useful tool for measuring extracellular concentrations of various endogenous molecules in the brain. After a microdialysis probe was inserted via a guide cannula into the anterior hypothalamic area (Ahy) of a rat, an artificial cerebral spinal fluid was perfused at 1 μl/min and the dialysate was collected every 30 min. HA concentration in the dialysate was determined by an automated LC system that involved the separation of free HA with a cation-exchange column (TSK gel SP-2SW, Toyo Soda, Japan) and detection with fluorimetry by a post-column derivatization technique using OPA reagent [34]. Fig. 3(A) and (B) express the results of circadian changes in HA release from the Ahy and locomotor activity of conscious, freely moving rats, respectively.

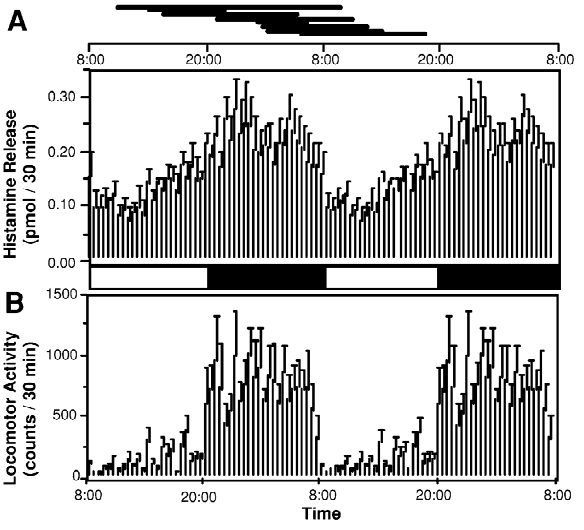

Fig. 3. Circadian change in histamine release from the anterior hypothalamic area (A) and locomotor activity (B) of conscious and freely moving rats. Mean and standard errors in 30-min periods are double-plotted. Horizontal bars at the top indicate sampling periods in individual experiments. Reproduced from Ref. [72].

4.3. Single cell measurements

By using the optimised post-column derivatization LC system described in the previous section in this review, Haaster et al. also measured endogenous HA after stimulating rat peritoneal mast cells with compound 48/80 in a suspension. They reported the total content of HA was 20 ± 3 μg/10^6 cells. Recently, the determination of the contents in single cell has come to be a trend because individual cells in biological samples are not equivalent to each other. The Wightman group [73] developed the method for determination of HA in single cell using a capillary LC system coupled with carbon fiber microelectrodes as the detection device. To separate released HA from single cell, the capillary column (40 cm) was fabricated using a 27 or 40 μm (I.D.) fused-silica capillary tube packed with 5 μm (I.D.) ODS particles. To make room for carbon fiber electrode placement at one end of the column, a frit was made at a position of 1 mm from the end of the capillary with 5 μm spherical silica particles by sintering in the flame of a match after tapping the end of the capillary into a pile of silica particles, and the particles were forced 1 mm into the column with a 25 μm tungsten wire. The flow-rate of the mobile phase (75 m*M* phosphoric acid–0.3 m*M* hexanesulfonic acid adjusted at pH 3) through the column was less than 60 nl/min. HA was detected at an oxidation potential of +1300 mV versus Ag/AgCl. Single cells were transferred to 300-nl microvials, followed by addition of 0.3 *M* $HClO_4$ to lyse the cell. Using optimized capillary LC, the HA content in the supernatant was analyzed. Although the analysis revealed a large cell-to-cell variation in the amount of HA in a rat peritoneal mast cell, the average HA level was 150 fmol.

In single cell analysis, the major hurdle is how to handle the single cell. The Ewing group [74] presented the method of introducing the single cell into a capillary tube where lysing of the single cell and tagging of analytes with derivatization reagent were carried out with on-column techniques. In their approach, the inlet segment of the capillary is used as the reaction chamber for cell lysing and derivatizing. Using this technique, they demonstrated the presence of catecholamines and their metabolites in single lymphocytes and extracts of T- and B-cell clones by use of CE with electrochemical detection. The Ewing group also measured levels of dopamine and five amino acids in single rat pheochromocytoma (PC12) cells with in-capillary derivatization CE coupled with LIF detection [75].

To avoid these complicated procedures, Oguri et al. [76] presented a method based on a different idea for measurements using single cells. This method involves a suspension of peritoneal mast cells (1×10^6 cells/ml of saline), collected from a male Wistar rat (8 weeks of age), which are directly introduced into the capillary tube from the anodic end by hydrostatic injection (at 25 cm height, for 2–20 s). When a high-voltage potential (25 kV) was applied to the capillary, which was already filled with a run buffer containing both a lysing reagent (SDS) and a derivatizing reagent (OPA/*N*-acetylcysteine, NAC), HA in the mast cells was detected at high-sensitivity levels without requiring any further procedures. During CE, the mast cells injected into the capillary were lysed with the lysing reagent, free HA released from the cell was labeled with the derivatizing reagent, and its derivative was electromigrated, separated and detected with a fluorescence detector in a fused-silica capillary. The run buffer used was a 20 m*M* phosphate–borate buffer (pH 10) containing 20 m*M* SDS, 2 m*M* OPA and 2 m*M* NAC. The SDS in the run buffer plays a dual role, both as a lysing reagent for the cell and as an additive of micelle formation for micellar electrokinetic chromatography (MEKC). This method was also examined with regard to the possibility of its use for determination of HA at the single mast cell level. The process of this determination using this method is illustrated in Fig. 4.

Fung et al. [77] developed a powerful and rapid detection system for the determination of HA and serotonin (5HT) levels in a single rat peritoneal mast cell. This method involved flow cytometry coupled with laser vaporization/ionization interface time-of-flight mass spectrometry (TOF-MS) employed as the detector. Flow cytometry is well-known as a useful device for analysis of single cells. A flowing stream of cells in a balanced salt solution is lined up in single file with the aid of a sheath liquid. When the cell passes the detection point, the cell contents are

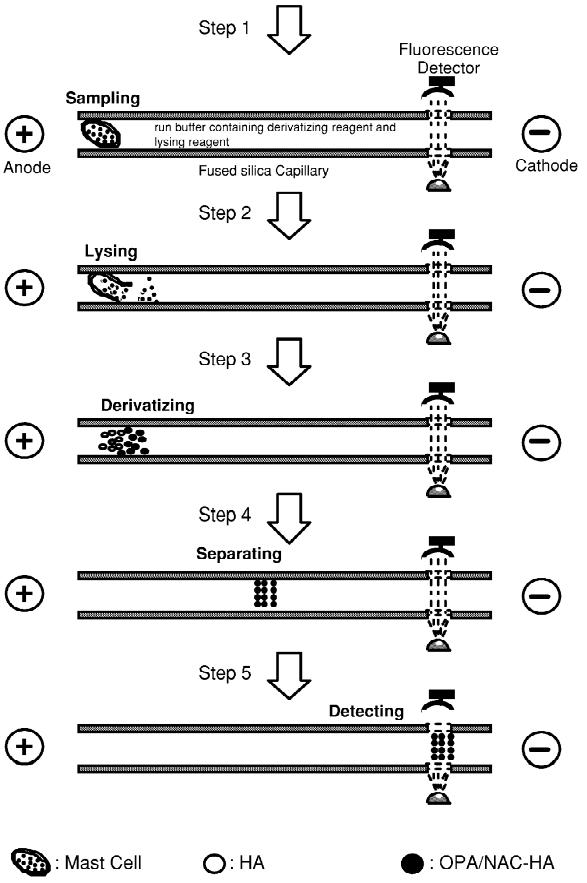

Fig. 4. Schematic representation of the method for determination of endogenous histamine levels in rat peritoneal mast cells. In step 1, the suspension of mast cells is directly injected hydrostatically into the capillary, which is filled with a run buffer consisting of 2 m*M* *o*-phthalaldehyde (OPA), 2 m*M* *N*-acetylcysteine (NAC), 20 m*M* sodium dodecyl sulfate (SDS), and 20 m*M* phosphate–borate buffer (pH 10). After that, in steps 2 and 3, a voltage potential of 25 kV was applied to the capillary. The cells were lysed with SDS in the run buffer (step 2), free histamine was liberated from the cells and was labeled with OPA/NAC, the derivatizing reagent in the run buffer (step 3); the derivative was then electromigrated and separated (step 4) and finally the derivative was detected with a fluorescence detector (step 5). Reproduced from Ref. [76].

vaporized and ionized by means of irradiating them with a pulse laser, and subsequently extracted into the flight tube of the TOF-MS. Using this system, they determined that the HA levels in each cell was accurately determined at a rate of 100 cells/5 min. According to their results, the average amount of HA in a single mast cell was found to be 0.33 ± 0.11 fmol.

5. Conclusions

Although many variations on established assay methods have been developed, and have been applied to biological samples in order to determine the levels of HA and its metabolites, every method has both merits and demerits. REA and EIA are two of the most standard methods adequate for routine use in the clinical laboratory due to their high detection sensitivities, but the use of radioisotopes or the preparation of antibody against HA or related compounds make researchers who have no facilities to handle these compounds reluctant to employ these methods. Further, it is hard to simultaneously determine HA and its metabolites using these methods. Chromatographic methods (GC and LC) have been developed as alternative microseparation techniques, which, on the one hand, possess the merit of being capable of making simultaneous determinations, but which, on the other hand, possess the demerit of requiring complicated procedures, viz: (1) pretreatment of samples in order to adequately remove undesirable compounds such as proteins, amino acids, etc.; (2) derivatization of analytes to convert them into volatile compounds and to enhance the detection selectivity or sensitivity for GC and LC, respectively.

Recently, the need to determination levels of HA and its related compounds in very small amounts of sample volume, such as in a limited area of an organ or in a single cell or granule, has increased. To perform such determinations, several ''hyphenation procedures'', i.e. the coupling of traditional assay techniques to a microdialysis device or modern detection devices, such as MS, LIF or electrochemical detectors, have been developed. Although these procedures have had limited applications until now, we strongly believe that modern separation techniques such as capillary LC, CEC, CE and μ-chip CE will become the standard methods employed, replacing REA, EIA or classical chromatographic methods for the determination of the levels of HA and its metabolites in biological samples. Finally, we

believe the use of these modern microseparation techniques will lead to the clarification of the various roles of HA and its metabolites in physiological and pathological processes.

6. Nomenclature

AAn	acetic anhydride
Ahy	anterior hypothalamic area
BGE	background electrolyte
CE	capillary electrophoresis
CEC	capillary electrochromatography
CI	chemical ionization
EGA	ethylene glycol adipate
DAO	diamine oxidase
DNTSA	2,6-dinitro-4-trifluoromethylbenzensulfonic acid
ECD	electron-capture detection
ECL	enterochromaffin-like
EI	electron impact
EIA	enzymeimmunoassay
FID	hydrogen flame ionization detection
GC	gas chromatography
IAA	imidazoleacetic acid
I.S.	internal standard
LC	liquid chromatography
HA	histamine
HFBAn	heptafluorobutyl anhydride
HDC	histidine decarboxylase
5HT	serotonin
IgE	immunoglobulin E
LIF	laser-induced fluorimetry
MS	mass spectrometer
MEKC	micellar electrokinetic chromatography
MHA	1-methylhistamine or N^{τ}-methylhistamine
MIAA	1-methylimidazoleacetic acid or N^{τ}-methylimidazoleacetic acid
NAC	*N*-acetylcysteine
NPD	nitrogen–phosphorus detector
ODS or C_{18}	octadecyl silane
OPA	*o*-phthalaldehyde
PFB-Br	pentafluorobenzyl bromide
PFPAn	pentafluoropropionyl anhydride
REA	radioenzymatic assay
SAR	seasonal allergic rhinitis
SDS	sodium dodecyl sulfate
SIM	selective ion-monitoring
TFAAn	trifluoroacetyl anhydride
Bis-TFMBO-Cl	3,5-bistrifluoromethylbenzoyl chloride
TOF-MS	time-of-flight mass spectrometry
UV	ultraviolet

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