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Short communication

High-performance liquid chromatographic method for the determination of histamine and 1-methylhistamine in biological buffers

Vivica von Vietinghoff, Gotthold Gäbel, Jörg R. Aschenbach *

Institute for Veterinary Physiology, University of Leipzig, An den Tierkliniken 7, D-04103 Leipzig, Germany

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Abstract

A method for the determination of histamine and its catabolite 1-methylhistamine (1-MH) was developed, using HPLC with fluorescence detection. Derivatization of both compounds occurred on-column with *o*-phthaldialdehyde dissolved in an alkaline borate buffer, followed by separation on a reversed phase C18 column. Histamine and 1-MH could be detected with comparable sensitivity (limit of quantification, 50 nM). The method was proven suitable to investigate catabolism of histamine by epithelia of pig colon. The method should be useful in research on histamine metabolism.

Keywords: High-performance liquid chromatography; Histamine; Histamine absorption; Histamine metabolism; Histamine release; Intestine; 1-Methylhistamine; Ussing chamber

1. Introduction

Histamine is a biogenic amine that can be released from different cell types to modulate body functions [1]. Additionally, histamine may enter the body from the lumen of the gut [2]. For both endogenous and exogenous histamine, catabolism is a major pathway to terminate its actions and to prevent intoxication [3–5].

A number of chromatographic methods are available to detect histamine in biological samples and to determine the production of its catabolite 1-methylhistamine (1-MH) [6]. Gas chromatography can be performed with high sensitivity if it is coupled to mass spectrometry (e.g. <1 nM or <1 pmol 1-MH in brain homogenates [7]). However, the equipment is very expensive. Capillary electrophoresis has also been used with similar detection limits (1.8 pmol for histamine, 0.8 pmol for 1-MH in standard solutions) but, because of the small injection volumes, it requires that histamine and 1-MH are concentrated to >20 μM in the samples [8]. In contrast, HPLC can use higher injection volumes and could potentially allow analyzing histamine and 1-MH in simple biological matrices without the need for extraction steps or sample concentration. A very sensitive method for the analysis of histamine (e.g. ≥1 nM histamine in aqueous solutions) had been developed by Tsikas et al. [9], which had been slightly modified by our group [10]. The goal of this study was to develop an HPLC-method that is suitable for routine analysis of both histamine and 1-MH, with minimal sample preparation and labour-saving on-column derivatization. We require such a method for a series of experiments on histamine catabolism in the porcine intestine. We proceeded from two published HPLC protocols that relied on *o*-phthaldialdehyde derivatization and fluorescence detection [11,12].

2. Experimental

2.1. Chemicals and reagents

Methanol, acetone and acetonitrile (HPLC-grade) were obtained from Roth (Karlsruhe, Germany), *o*-phthaldialdehyde (OPA) and histamine dihydrochloride were supplied by Fluka (Buchs, Switzerland). 1-MH was purchased from Sigma (Taufkirchen, Germany). Carbogen (95% O_2/5% CO_2) was supplied by Messer Griesheim (Krefeld, Germany). All other chemicals were obtained either from VWR (Darmstadt, Germany) or from Sigma.

* Corresponding author. Tel.: +49 341 97 38076; fax: +49 341 97 38097.
E-mail address: aschenb@rz.uni-leipzig.de (J.R. Aschenbach).

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2.2. Standards and calibration curves

Stock solutions of histamine and 1-MH were prepared separately by dissolving 10 mM of each substance in 0.1 M HCl. They were stored in aliquots at −80 °C. To prepare calibration curves, histamine and 1-MH were diluted together in potassium phosphate buffer (25 mM; pH 7.4) or buffer from Ussing chamber experiments. Standards for calibration of Ussing chamber experiments were prepared on the same day as the experiments were conducted. Thus, standards and samples were treated in the same way, including storage (at −80 °C) and thawing. Two calibration curves (0.05–0.5 μM) were included in the determination of each sample batch from Ussing chambers for in process control.

During validation experiments, a set of standard dilutions was run and evaluated five times on one day to determine intra-day variability. The same set of standard dilutions was analyzed on five different days within a two-week period for inter-day variability.

2.3. Chromatographic separation and detection

Histamine and 1-MH were determined with HPLC equipment from Waters Corporation (Milford, MA, USA), using the software Millennium^{TM} 2010. A volume of 110 μL of each sample taken from the Ussing chamber experiment or of any calibration standard was mixed with 10 μL of *N*-acetyl-L-cysteine (NAC) solution (100 mg NAC ad 1 ml 3 M NaOH) and put in an autosampler (Waters^{TM} 717 Autosampler) cooled to 4 °C. Volumes of 100 μL were injected automatically onto the column EC 125/4 Reprosil-Pur C18 AQ, 5 μm; guard column: CC 10/4 Reprosil-Pur C18 AQ, 5 μm (both from Trentec-Analysentechnik, Gerlingen, Germany). Columns were thermostated to 25 °C. The mobile phase contained 30 mM $Na_2Ba_4O_7$ in methanol–water (40/60; v/v) and 0.2 mM OPA. The eluent was kept in a dark box during analyses and in a refrigerator at 4 °C between analyses to prevent OPA degradation. After separation at a flow rate of 1.5 ml/min (Waters^{TM} 510 HPLC Pump), the derivatives of histamine and 1-MH were detected with a fluorescence detector (Waters^{TM} 470 Scanning Fluorescence Detector; excitation: 350 nm; emission: 450 nm). Run time was 13 min.

2.4. Ussing chamber samples

Fattening pigs were anaesthetised (azaperone, 2 mg/kg; thiamylal, 17 mg/kg) and slaughtered. Intestinal segments of the proximal colon were removed and the epithelia were separated from the muscular and serosal layers with a slide strip technique [13]. Epithelia were mounted between the two halves of Ussing chambers, following the protocol of Gäbel et al. [13]. An area of 1.1 cm^2 on the mucosal (i.e. lumen-directed) and serosal (i.e. blood-directed) sides were incubated separately in 12 ml of buffer solution containing (in mM): NaCl 105, KCl 5, 3-(*N*-morpholino)propanesulfonic acid (MOPS) 5, $CaCl_2$ 1.8, $MgCl_2$ 1, $NaHCO_3$ 25, Na_2HPO_4 1, D-glucose 10, sodium propionate 5, sodium butyrate 2, H_3PO_4 2, and enrofloxacin 0.03. Incubation buffer had an initial pH of 7.35–7.40 and was agitated constantly by gassing with 95% O_2/5% CO_2.

Consecutive samples were taken from the serosal solution to determine the serosal appearance of histamine and 1-MH. Some epithelia received exogenous histamine to a final concentration of 100 μM at the mucosal side, and some epithelia were additionally treated with aminoguanidine (100 μM, bilaterally). All buffer samples were frozen at −80 °C immediately after sampling.

2.5. Presentation of results and statistical analysis

If not stated otherwise, data are presented as means ± S.D. Statistical evaluation of data included assessment of differences between means (ANOVA) and simple linear regression estimates. Statistics was performed using the software Jandel SigmaStat version 2.0 (Systat, Erkrath, Germany).

All experimental procedures were in accordance with the German laws and were approved by the appropriate authority, the Regierungspräsidium Leipzig (AZ 24-9162.11).

3. Results and discussion

3.1. Chromatographic separation and detection

Initially, we tested the method of Saito et al. [11], which uses on-column fluorescence derivatization with OPA to detect 1-MH and histamine. However, it was not possible to achieve a stable baseline with the original chromatographic protocol [11]. OPA dissolved together with NAC in H_2O–acetonitrile; (86/14; v/v) produced by itself, i.e. without any added primary amine, a highly intense fluorescence signal. This high background fluorescence was remarkably reduced after replacing acetonitrile by methanol. However, the mixing of OPA and NAC in the mobile phase still resulted in a continuous increase of baseline without plateau, thus masking smaller peaks. Therefore, NAC was omitted from the mobile phase and added directly to the samples. This separation of OPA and NAC had no negative effect on derivatization efficiency, e.g. no peak broadening or reduction in signal occurred. The amount of OPA within the mobile phase could be reduced from 1 mM [11] to 0.2 mM without any changes in peak shape and peak area. Thus, reproducible chromatographic separation of histamine and 1-MH was obtained with 0.2 mM OPA dissolved in a methanol–water (40/60, v/v) solution containing 30 mM $Na_2Ba_4O_7$. The pH of this mobile phase was pH 9.2–9.4. It was not necessary to adjust the pH, as already Saito et al. [11] had demonstrated that any pH above 9 leads to reproducible and symmetrical peaks.

3.2. Validation in phosphate buffer

Validation was performed initially in phosphate buffer at concentrations between 0.05 and 0.5 μM of both histamine and 1-MH (Tables 1 and 2). At all concentrations, histamine and 1-MH were clearly separated from each other and could be detected with high specificity. Fig. 1A shows an injection of buffer solution containing NAC (blank). Due to the interaction

Table 1
Precision and accuracy of histamine determination

Nominal concentration (μM)	Intra-day assay			Inter-day assay		
	Measured concentration[a] (μM)	Precision R.S.D.[b] (%)	Accuracy R.E.[c] (%)	Measured concentration[a] (μM)	Precision R.S.D.[b] (%)	Accuracy R.E.[c] (%)
Phosphate buffer [calibration: $y_H = (50 \pm 56) + (19{,}684 \pm 182)x_H$; $r = 1.000$; $n = 9$][d]						
0.05	0.051 ± 0.003	5.29	1.39	0.055 ± 0.006	11.48	10.15
0.1	0.096 ± 0.011	11.70	−3.91	0.093 ± 0.012	12.67	−6.68
0.2	0.213 ± 0.008	3.75	6.45	0.193 ± 0.024	12.59	−3.32
0.3	0.328 ± 0.008	2.37	9.46	0.284 ± 0.040	13.94	−5.46
0.4	0.437 ± 0.019	4.26	9.31	0.378 ± 0.047	12.39	−5.50
0.5	0.536 ± 0.037	6.94	7.17	0.474 ± 0.067	14.18	−5.19
Phosphate buffer [calibration: $y_H = (5{,}020 \pm 2{,}930) + (17{,}057 \pm 353)x_H$; $r = 0.999$; $n = 5$][d]						
1.25	ND[e]			1.04 ± 0.13	12.43	−16.87
2.5	ND			2.38 ± 0.24	10.00	−4.70
5.0	ND			5.29 ± 0.38	7.15	5.84
7.5	ND			7.74 ± 0.85	11.00	3.19
10.0	ND			9.93 ± 0.70	7.09	−0.70
15.0	ND			14.87 ± 0.77	5.21	−0.89
Buffer from Ussing chambers [calibration: $y_H = (1{,}432 \pm 601) + (16{,}498 \pm 72)x_H$; $r = 1.000$; $n = 9$][d]						
1.25	1.26 ± 0.08	6.60	0.49	1.28 ± 0.09	6.95	2.14
2.5	2.45 ± 0.02	0.98	−2.09	2.58 ± 0.20	7.94	2.90
5.0	4.87 ± 0.06	1.23	−2.66	5.15 ± 0.42	8.21	2.91
7.5	7.15 ± 0.17	2.39	−4.64	7.66 ± 0.63	8.20	2.18
10.0	9.75 ± 0.14	1.45	−2.51	10.28 ± 0.91	8.87	2.79
15.0	14.67 ± 0.34	2.34	−2.20	15.43 ± 1.47	9.55	2.84

[a] Values represent means ± S.D.; $n = 5$.
[b] Relative standard deviation.
[c] Relative error.
[d] y_H: peak area (μV*s); x_H: histamine concentration (μM).
[e] Not determined.

Table 2
Precision and accuracy of 1-MH determination

Nominal concentration (μM)	Intra-day assay			Inter-day assay		
	Measured concentration[a] (μM)	Precision R.S.D.[b] (%)	Accuracy R.E.[c] (%)	Measured concentration[a] (μM)	Precision R.S.D.[b] (%)	Accuracy R.E.[c] (%)
Phosphate buffer [calibration: $y_{1\text{-MH}} = (83 \pm 66) + (20{,}162 \pm 218)x_{1\text{-MH}}$; $r = 1.000$; $n = 9$][d]						
0.05	0.051 ± 0.002	4.57	2.60	0.054 ± 0.003	6.15	7.66
0.1	0.093 ± 0.006	6.55	−6.62	0.092 ± 0.008	8.42	−7.54
0.2	0.201 ± 0.009	4.53	0.68	0.202 ± 0.012	5.93	0.91
0.3	0.302 ± 0.010	3.33	0.82	0.306 ± 0.026	8.41	2.16
0.4	0.401 ± 0.029	7.31	0.15	0.407 ± 0.023	5.67	1.83
0.5	0.500 ± 0.015	2.98	−0.07	0.497 ± 0.041	8.19	−0.62
Phosphate buffer [calibration: $y_{1\text{-MH}} = (4{,}425 \pm 2{,}590) + (17{,}305 \pm 311)x_{1\text{-MH}}$; $r = 0.999$; $n = 5$][d]						
1.25	ND[e]			1.08 ± 0.14	13.38	−13.67
2.5	ND			2.40 ± 0.26	10.95	−3.88
5.0	ND			5.23 ± 0.38	7.31	4.63
7.5	ND			7.74 ± 0.68	8.76	3.18
10.0	ND			9.89 ± 0.57	5.80	−1.08
15.0	ND			14.91 ± 0.65	4.34	−0.63
Buffer from Ussing chambers [calibration: $y_{1\text{-MH}} = (1{,}432 \pm 601) + (16{,}498 \pm 72)x_{1\text{-MH}}$; $r = 1.000$; $n = 9$][d]						
1.25	1.26 ± 0.06	4.37	0.82	1.28 ± 0.10	7.50	2.04
2.5	2.45 ± 0.02	0.63	−1.96	2.55 ± 0.22	8.73	1.87
5.0	4.98 ± 0.05	0.98	−0.44	5.10 ± 0.41	7.96	1.91
7.5	7.27 ± 0.13	1.82	−3.08	7.57 ± 0.66	8.77	0.93
10.0	9.87 ± 0.06	0.59	−1.34	10.10 ± 0.83	8.21	1.04
15.0	14.85 ± 0.19	1.28	−0.97	15.87 ± 1.44	9.16	1.59

[a] Values represent means ± S.D.; $n = 5$.
[b] Relative standard deviation.
[c] Relative error.
[d] $y_{1\text{-MH}}$: peak area (μV*s); $x_{1\text{-MH}}$: 1-MH concentration (μM).
[e] Not determined.

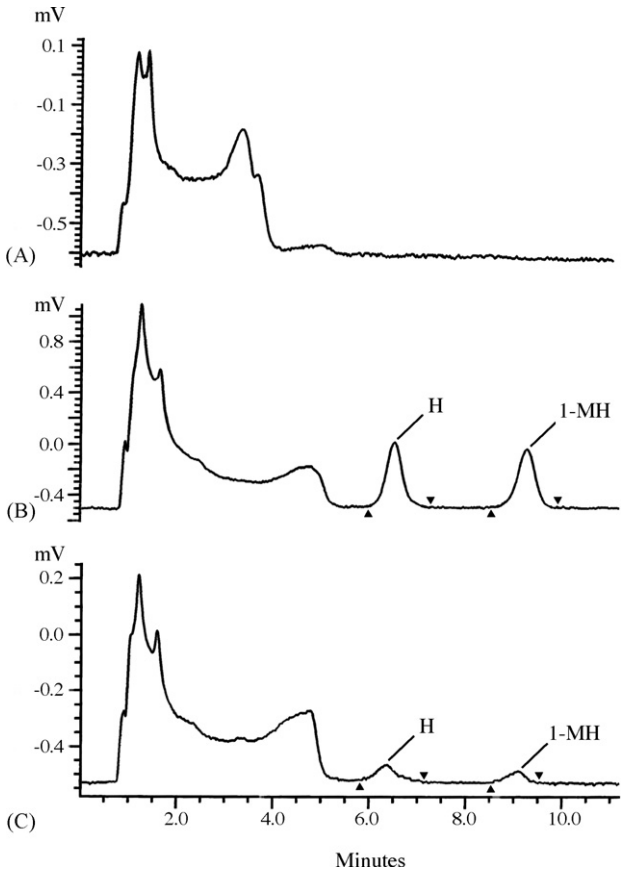

Fig. 1. HPLC-chromatograms from phosphate buffer standards. (A) Blank sample. (B) Peaks of histamine (H) and 1-MH at a concentration of 0.5 μM each. (C) Peaks at the LOQ (0.05 μM) for histamine and 1-MH.

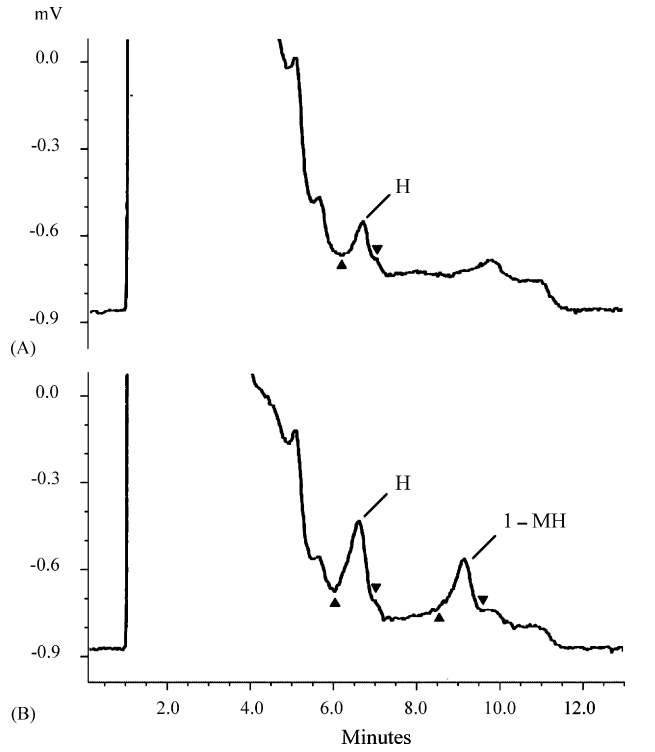

Fig. 2. HPLC-chromatograms from Ussing chamber solution. (A) Injection of an authentic sample of Ussing chamber solution drawn to measure serosal release 4.5 h after start of incubation. Only histamine showed a distinct peak above detection limit. (B) Chromatogram of the same sample after spiking with 0.5 μM of both histamine and 1-MH. The increases in peak area corresponded to 0.491 and 0.568 μM for histamine and 1-MH, respectively.

of OPA and NAC, a broad peak occurred at the beginning of the chromatogram. After 5.5 min, however, no interfering endogenous peaks were detected. Thus, histamine and 1-MH could be identified with good baseline separation thereafter (Fig. 1B and C). Retention times were 6.43 ± 0.05 and 9.22 ± 0.10 min for histamine and 1-MH, respectively ($n = 9$). The theoretical LOQ (signal-to-noise ratio, 10:1) was at a concentration of 0.05 μM for both amines (Fig. 1C). This is an approximately 20-fold increase in sensitivity compared to the original method of Saito et al. [11].

Chromatographic separation showed excellent linearity over the tested concentration range ($r > 0.99$ for each individual calibration curve). The slopes of the regression lines were not different for histamine and 1-MH (Tables 1 and 2), indicating an equal efficiency of derivatization and chromatographic separation of both compounds. The slopes of the regression lines did not change when additionally implementing concentrations of 5 and 50 μM into the regression analyses (data not shown). It is therefore justified to conclude that the method can be applied to analyze histamine and 1-MH concentrations in a phosphate buffer over a minimum of three orders of magnitude, i.e. from 0.05 to 50 μM. These analyses can be performed with acceptable precision and accuracy as shown in Tables 1 and 2 for both intra- and inter-day assay comparisons.

3.3. Ussing chamber experiments

Our group requires an easy-to-use method with high throughput capacity for a series of studies on histamine absorption and catabolism in intestinal epithelia [14].

We therefore tested the applicability of the new HPLC protocol to buffer samples from Ussing chamber experiments. Parallel validation experiments with Ussing chamber solutions and phosphate buffer gave almost identical results with respect to linearity, precision and accuracy (Tables 1 and 2). LOQ was also comparable with short incubations of epithelia (<1 h). With longer incubations, baseline separation and LOQ started to get slightly compromised. Fig. 2 shows a chromatogram from a buffer sample drawn after an extended incubation period of 4.5 h. Despite some compromise in baseline separation in these very late samples, spiked histamine and 1-MH could be quantified very accurately (Fig. 2).

Prolonged incubations were avoided with the final sets of samples to proof routine applicability, i.e. samples were drawn 1.5 and 2.5 h after mounting of epithelia. We monitored the appearance of histamine and 1-MH on the serosal side under control conditions or during simulation of a luminal histamine burden of 100 μM in the absence or presence of aminoguanidine. Histamine appearance was measurable for every pig in each group. 1-MH concentration was more variable and below the detection limit in four animals of the control group and even three animals of the group receiving 100 μM histamine mucosally.

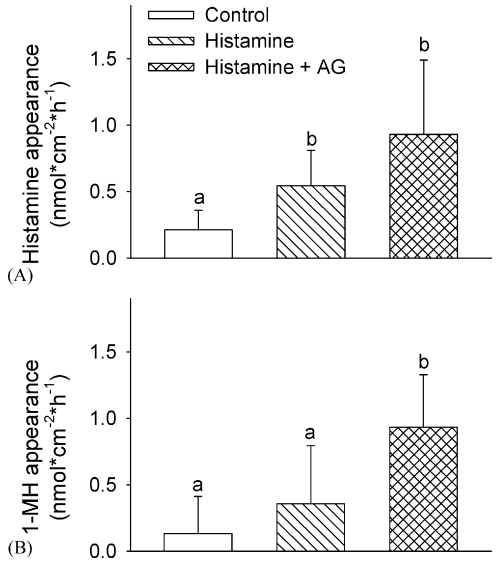

Fig. 3. Appearance of (A) histamine and (B) 1-MH on the serosal side of isolated colonic epithelia of pigs in Ussing chambers. Appearance under control conditions is compared with appearance during simulation of a luminal histamine burden (100 μM). Some epithelia were additionally treated with 100 μM aminoguanidine on both sides (AG). Data represent means and S.D. from colonic epithelia of six animals. [a,b]Columns within one panel that do not share a common letter are different ($P < 0.05$).

After combined application of histamine and aminoguanidine, however, 1-MH was measurable in all buffer samples. A comparison of treatments (Fig. 3) showed that a luminal histamine burden increased the appearance of histamine on the serosal side ($P < 0.05$), which can be interpreted as histamine absorption. A blockade of diamine oxidase by aminoguanidine [4,5] additionally increased the appearance of 1-MH ($P < 0.05$), indicating that 1-MH is a main substrate of diamine oxidase in the porcine colon.

4. Conclusion

The present HPLC protocol provides a method with acceptable accuracy and precision for the detection of histamine and 1-MH in biological buffers. The main advantage of the method is the very simple, fast, cheap and labour-saving protocol, which makes it especially suitable for high throughput applications. While we were able to apply the method without any extraction procedure, it also bears the potential to be combined with established methods for histamine and 1-MH extraction where the analyte is recovered in aqueous solutions. Therefore, this protocol could potentially be useful in amine analysis beyond the here described application.

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