Journal of Chromatography, 383 (1986) 19—26
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3304

ELECTROCHEMICAL DETERMINATION OF HISTAMINE DERIVATIZED WITH *o*-PHTHALALDEHYDE AND 2-MERCAPTOETHANOL

LASZLO G. HARSING, Jr.*

Institute of Experimental Medicine, Hungarian Academy of Sciences, Szigony u. 43, P.O. Box 67, H-1450 Budapest (Hungary)

HIDEO NAGASHIMA

Department of Anesthesiology, Albert Einstein College of Medicine, Montefiore Hospital and Medical Center, Bronx, NY 10467 (U.S.A.)

E. SYLVESTER VIZI

Institute of Experimental Medicine, Hungarian Academy of Sciences, Szigony u. 43, P.O. Box 67, H-1450 Budapest (Hungary)

and

DERYCK DUNCALF

Department of Anesthesiology, Albert Einstein College of Medicine, Montefiore Hospital and Medical Center, Bronx, NY 10467 (U.S.A.)

(First received February 5th, 1986; revised manuscript received June 19th, 1986)

SUMMARY

High-performance liquid chromatography coupled with electrochemical detection was used to determine histamine following precolumn derivatization with *o*-phthalaldehyde (OPA) and 2-mercaptoethanol. The isoindole derivative which is obtained as reaction product was electrochemically active at a moderate potential (peak potential +0.4 V). Direct oxidation of histamine required a much higher potential (peak potential +1.05 V) and was of no practical use. No electrochemical signal was observed for the reaction product of histamine with OPA. Changing the pH of the mobile phase had little effect on the electrochemical response of the isoindole derivative of histamine, which was well separated from analogous derivatives of methylated histamines, mono- and polyamines and amino acids by isocratic elution from a reversed-phase column. An example of a practical application of the method to the estimation of histamine in rat brain is presented.

INTRODUCTION

High-performance liquid chromatography (HPLC) coupled with electrochemical detection has been widely used for the determination of biogenic

0378-4347/86/$03.50

amines and their metabolites. The success of electrochemical methods for detection of aromatic and indoleamines is due to the moderate electrochemical potentials required for their oxidation [1]. However, many biologically important compounds such as histamine, polyamines, most of the amino acids and peptides possess no structural features suitable for detection within the currently useful potential range. Precolumn derivatization with *o*-phthalaldehyde (OPA) in the presence of 2-mercaptoethanol (2-ME) renders isoindole derivatives of compounds containing primary amino groups [2] which can be detected both by fluorimetry and by electrochemical oxidation [3–5].

The condensation of OPA alone with certain primary amines [6, 7], polyamines [8, 9] and amino acids [10, 11] is known to produce fluorescent derivatives as well, however, with not defined structures. One of the most common applications of derivatization with OPA is for the fluorometric determination of histamine [12, 13]. It was our aim to study whether histamine and its derivatives could be determined in biological material by electrochemical detection more sensitively than by fluorimetry. Condensation with OPA/2-ME and subsequent isocratic separation of the reaction products by HPLC resulted in a potentially useful procedure with improved sensitivity.

EXPERIMENTAL

Chemicals

Chemicals were purchased from the following sources: histamine · 2HCl, spermine · 4HCl, spermidine · 3HCl, imidazole, norepinephrine · HCl, epinephrine bitartrate, dopamine · HCl, serotonin creatinine sulfate, 1-amino acids, *o*-phthalaldehyde, 2-mercaptoethanol from Sigma (St. Louis, MO, U.S.A.); N^{α}-methylhistamine · 2HCl, 1-methylhistamine (N-tele-methylhistamine) · 2HCl, 3-methylhistamine (N-pros-methylhistamine) · 2HCl from Calbiochem-Behring (San Diego, CA, U.S.A.); 4-methylhistamine · 2HCl from Smith, Kline and French Labs. (U.K.); 4-imidazoleacetic acid · HCl from Aldrich (Milwaukee, WI, U.S.A.); Amberlite CG-50 (100–200 mesh) from Mallinckrodt (Paris, KY, U.S.A.); [2,5-^{3}H]histamine · 2HCl (specific activity 1.92 TBq/mmol) from Amersham (Arlington Heights, IL, U.S.A.). Methanol and acetonitrile were HPLC grade, all other chemicals were reagent grade.

Chromatography

Isocratic liquid chromatographic experiments were performed using two different systems. For non-derivatized histamine: Biotronik HPLC system (Biotronik Wissenschaftliche Geräte, Frankfurt am Main, F.R.G.) consisting of a BT 3020 high-pressure pump with pulse dampener, Rheodyne 7125 injector with 20-μl sample loop, 150 × 4.6 mm Nucleosil C_{18} reversed-phase column, 5-μm particles (Bischoff Analysentechnik). An ESA (Environmental Sciences Associates, Bedford, MA, U.S.A.) electrochemical detector with a Model 5020 guard cell (at +1.0 V) and a Model 5010 dual-electrode analytical cell with porous graphite electrodes at +0.8 and +1.05 V, respectively, versus palladium reference electrodes. Signals of the electrochemical detector were monitored on a two-channel chart recorder. For direct oxidation of histamine the following mobile phase was used: 0.01 *M* NaH_2PO_4, pH adjusted to 6.5 with 1

M sodium hydroxide, 21% methanol and 0.2 m*M* sodium dodecyl sulfate; flow-rate 1.0 ml/min.

The HPLC apparatus used for the determination of the histamine-OPA/2-ME derivative was a Waters HPLC system (Waters Assoc., Milford, MA, U.S.A.) consisting of an M-45 solvent delivery system with pulse dampener, U6K universal injector, 150 × 4.6 mm Nucleosil C_{18} reversed-phase column, 5-μm particles (Alltech Assoc., Deerfield, IL, U.S.A.), an LC 4B amperometric detector (Bioanalytical Systems, West Lafayette, IN, U.S.A.) connected to a glassy carbon (TL-5) working electrode at +0.5 V versus an Ag/AgCl reference electrode; recording of signals was done using a Waters 750 data module. The mobile phase used for the separations of the histamine isoindole derivative consisted of 0.07 *M* Na_2HPO_4—citric acid buffer, pH 4.5, containing 23% acetonitrile and 0.26 m*M* Na_2EDTA. The flow-rate was 1 ml/min and the temperature was ambient. For the determination of the pH dependence of oxidation of histamine-OPA/2-ME derivative the same mobile phase was used except that the pH was adjusted to 7. Mobile phases were prepared with water obtained from a Millipore water purification system and filtered through 0.2-μm Millipore membranes and degassed prior to use.

Derivatization of histamine with OPA/2-ME

Sodium tetraborate solution (0.1 *M*) was prepared from 0.4 *M* boric acid by adjusting its pH to 9.5 with 1 *M* sodium hydroxide [3, 11]. Aliquots (2.5 ml) of sodium tetraborate solution were mixed with 2.5 ml of water and 5 ml of methanol. OPA (0.25%, w/v) and 2-ME (0.25%, v/v) were dissolved in methanol and kept separately at room temperature in light-protected bottles. Solutions of histamine and all other compounds contained 10 mg free base per ml of 0.1 *M* hydrochloric acid (with 0.1% Na_2EDTA). The compounds, which were kept refrigerated, were diluted before use in 0.1 *M* hydrochloric acid.

For derivatization of histamine (or other compounds), reagents were mixed in the following order: 100 μl sodium tetraborate—methanol buffer were added to 10 μl OPA. This solution was mixed with 10 μl 2-ME and finally 10 μl histamine solution (0.005—2.0 μg/ml) were added [10]. The mixture was protected from light, stirred for exactly 1 min, and an aliquot of the reaction mixture was injected into the HPLC system 2 min after the reaction was started. In the case of biological samples, sodium tetraborate—methanol buffer was replaced by mobile phase made alkaline (pH 11.5) with 2 *M* sodium hydroxide. Condensation with OPA/2-ME was carried out as described above.

Extraction of histamine from brain tissue

Rats were decapitated and frontal cortex, striatum and hypothalamus were dissected from the brain according to the method of Glowinski and Iversen [14]. The tissues were weighed and homogenized by ultrasound in 400 μl of 0.2 *M* perchloric acid containing 0.1% Na_2EDTA. 3-Methylhistamine (50 ng) was added as internal standard and the samples were centrifuged. The pH of the supernatant was adjusted to 7.5 by addition of 50 μl of 2 *M* sodium hydroxide and 1.0 ml of 1.5 *M* Tris—HCl buffer, pH 7.5.

Amberlite CG-50 resin was prepared as described by Oates et al. [15] and histamine was isolated using the chromatographic procedure of Robert et al.

[16]. By adding [^{3}H]histamine ($3.7 \cdot 10^3$ Bq), the recovery of histamine from the cation-exchange resin was demonstrated to be 70.5 ± 3.2% (mean ± S.D., n = 4). Histamine was eluted from the resin with 2 × 1 ml of 0.5 *M* hydrochloric acid. After evaporation in vacuo the residues were redissolved in 200 µl mobile phase made alkaline (pH 11.5) with 2 *M* sodium hydroxide. An aliquot (50—150 µl) of the samples was mixed with OPA/2-ME as described above and injected into the HPLC column. External standards containing histamine (0—80 ng) and internal standard (50 ng of 3-methylhistamine) were handled in the same way as brain extracts and 50 µl of the redissolved samples were injected into the HPLC system.

RESULTS AND DISCUSSION

For characterization of the electrochemical properties of histamine and the histamine-OPA/2-ME derivative, chromatographically assisted hydrodynamic voltammograms were generated. As shown in Fig. 1, histamine is oxidized in a potential range of +0.8—1.05 V. The half-wave potential ($E_{½}$) was +0.89 V and the peak potential (E_p) +1.05 V.

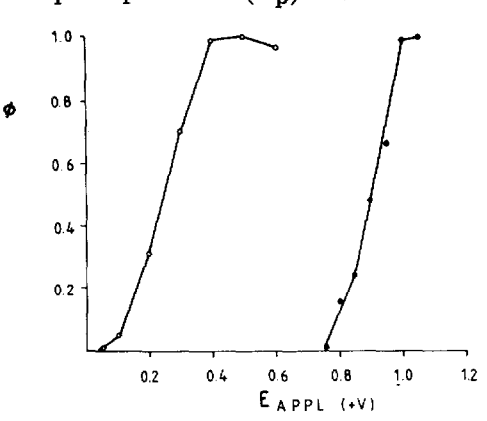

Fig. 1. Chromatographically assisted hydrodynamic voltammograms for histamine (•) and histamine-OPA/2-ME derivative (o). Abscissa: applied oxidation potential, ordinate: Φ = ratio of response at a particular potential to maximal response. For details see Experimental.

Histamine has two potential sites suited to electrochemical oxidation, namely the nitrogen atoms in positions 1 and 3 of the imidazole ring [17]. Using the dual-electrochemical cell in screen mode by adjusting the electrodes to +0.8 and +1.05 V, respectively, resulted in an undesirably high background current even when a guard cell was used for oxidation of impurities presumably present in the mobile phase or when the salt concentration in the mobile phase was reduced to a low level. Owing to the high background current an unstable baseline was observed. No attempts were therefore made to determine histamine in biological samples using this method.

Using a glassy carbon electrode of the TL-5 type electrochemical signals of the histamine-OPA/2-ME derivative were obtained in a potential range of +0.1—0.6 V (Figs. 1 and 2). The $E_{½}$ and E_p values for oxidation of the isoindole were +0.24 and +0.4 V, respectively. The maximal response at pH 7

was close to that observed at pH 4.5, indicating that the oxidation potential is not much dependent on pH. Determinations were carried out at +0.5 V. None of the technical difficulties arising in the case of direct oxidation of histamine was observed. In contrast with the isoindole, formed by reaction of histamine with OPA/2-ME, the product of histamine and OPA is not oxidized at +0.5 V (Fig. 2).

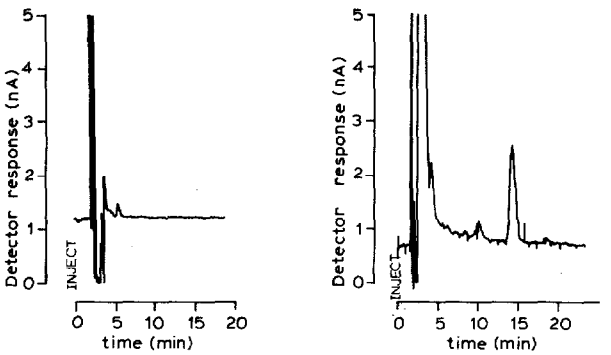

Fig. 2. HPLC profiles of histamine derivatized with OPA (left panel) and OPA/2-ME (right panel). Histamine (5 ng) was derivatized with either OPA or OPA/2-ME as described in Experimental. A small peak with a retention time of 10.5 min also appeared in the HPLC profile of histamine derivatized with OPA/2-ME.

Almost all authors have pointed out the labile nature of the histamine-OPA/2-ME reaction product [12, 13, 15, 16, 18]. Hydrolysis of isoindole at position 1 is perhaps responsible for the breakdown of OPA/2-ME derivatives [2]. The isoindole is more stable if the pH is reduced [12, 19]. Extraction into ethyl acetate also stabilizes the condensation products [6]. We found a maximal electrochemical response 2 min after starting the derivatization and a 33% decomposition of the histamine derivative was observed during a 60-min

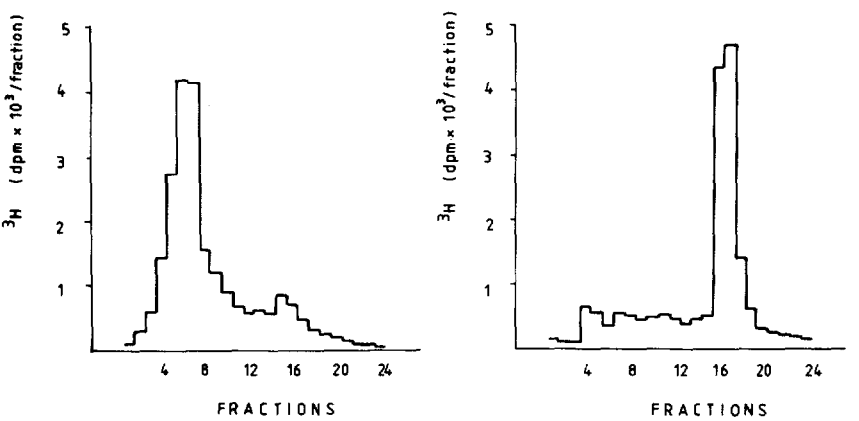

Fig. 3. HPLC elution profile of [^{3}H]histamine derivatized with OPA (left panel) and OPA/2-ME (right panel). In the absence of 2-ME, the radioactivity was detected in the fraction eluted close to the solvent front. [^{3}H]Histamine (100 nCi) was added to the derivatizing mixture, 1 ml/min fractions were collected and the radioactivity in the fractions was determined using a Packard Tri-Carb scintillation system (Model 4530). HPLC conditions and derivatization were as described in Experimental.

period. Therefore, care was taken to ensure that the reaction mixture was injected onto the HPLC column exactly 2 min after the reaction was started. Addition of 10 μl of 1 *M* hydrochloric acid to the reaction mixture at the end of the reaction lowered the pH to 2 and reduced the peak height. This procedure was therefore not adopted. The weakly acidic mobile phase, however, seems to stabilize the histamine-OPA/2-ME derivative as it traverses the HPLC system [16, 20].

Derivatization of [^{3}H]histamine with OPA/2-ME resulted in quantitative conversion of the radioactivity into the reaction product (91.3 ± 5.0%, mean ± S.D., n = 4, Fig. 3). [^{3}H]Histamine eluted from the HPLC column in a different position when it was derivatized with OPA alone (Fig. 3). The reaction product of histamine with OPA/2-ME was well resolved on the Nucleosil C_{18} column from other amine and amino acid derivatives and even from various N-methylhistamines (Table I), using 0.07 *M* Na_2HPO_4—citric acid

TABLE I

CAPACITY FACTORS (k') OF VARIOUS COMPOUNDS DERIVATIZED WITH o-PHTHALALDEHYDE AND 2-MERCAPTOETHANOL

Capacity factor (k') was defined as $(V_t - V_0)/V_0$, where V_t = retention volume of compound tested and V_0 = void volume. For HPLC conditions see Experimental. N.D. = not detectable.

Compound	Capacity factor	Compound	Capacity factor
Histamine	5.36	Glutamic acid	1.80
4-Methylhistamine	6.26	Glycine	1.90
3-Methylhistamine	7.01	Arginine	1.95
1-Methylhistamine	7.91	Threonine	2.40
N^{α}-Methylhistamine	N.D.	Alanine	3.10
4-Imidazoleacetic acid	N.D.	Tyrosine	3.17
Imidazole	N.D.	Asparagine	12.80
Norepinephrine	>15	Valine	13.33
Epinephrine	N.D.	Methionine	14.96
Dopamine	>15	Cystein	>15
Serotonin	>15	Glutamine	>15
Spermine	>15	Isoleucine	>15
Spermidine	>15	Leucine	>15
Aspartic acid	1.20	Lysine	>15
Histidine	1.20	Phenylalanine	>15
Serine	1.50	Tryptophan	>15

TABLE II

HISTAMINE CONTENT IN RAT BRAIN AREAS

Histamine was extracted and derivatized with OPA/2-ME as described in Experimental. For HPLC conditions see Experimental.

Brain area	Histamine content (mean ± S.D., n = 5) (ng/g)
Hypothalamus	334.3 ± 43.8
Striatum	77.9 ± 15.4
Frontal cortex	39.1 ± 14.9

buffer, pH 4.5, 23% acetonitrile and 0.26 mmol/l Na_2EDTA as eluent. Separations of histamine-OPA and histamine-OPA/2-ME reaction products were previously reported, using LiChrosorb, Nucleosil and μBondapak phenyl or CN columns [6, 7, 19, 21] and μBondapak C_{18} columns used in the reversed-phase ion-pairing conditions [16, 18]. Since norepinephrine, epinephrine, dopamine and serotonin can be directly oxidized at +0.5 V [1], the capacity factors of these amines were also determined. Owing to the high acetonitrile concentration, these non-derivatized biogenic amines eluted close to the solvent front. The detection limit for histamine in this assay was 50 pg at a signal-to-noise ratio of 3:1. In an other series of experiments 5 ng histamine-OPT/2-ME was repeatedly injected to determine the reproducibility of the derivatization. The coefficients of variation calculated for peak height and retention time were 1.0 and 0.8%, respectively (n = 5).

A linear calibration curve was obtained when the ratios of the peak heights for histamine to those of 3-methylhistamine were plotted against the amount of histamine injected. The histamine content in the brain samples was determined from the standard curve. Since 3-methylhistamine is not present in rat brain extracts [22], 3-methylhistamine as well as 4-methylhistamine may be used as internal standard. Histamine is unevenly distributed in brain with the highest level in the hypothalamus (Table II). Our values are comparable to those reported earlier [23, 24]. The proposed method allows the simultaneous determination of histamine and 1-methylhistamine, the main metabolite of histamine in brain tissue which is also a marker of histamine turnover rate [25]. Acidic metabolites of histamine (4-imidazoleacetic acid, 1-methyl-4-imidazoleacetic acid) cannot, however, be detected by precolumn off-line derivatization with OPA/2-ME. The sensitivity of this method is such that the histamine content of different regions of the brain may be determined in individual rats whereas measurements with fluorometric assays may require tissue pooling from several animals [23].

REFERENCES

1 I.N. Mefford, J. Neurosci. Methods, 3 (1981) 207.
2 S.S. Simons, Jr. and D.F. Johnson, J. Am. Chem. Soc., 98 (1976) 7098.
3 M.H. Joseph and Ph. Davies, J. Chromatogr., 277 (1983) 125.
4 L.A. Allison, G.S. Mayer and R.E. Shoup, Anal Chem., 56 (1984) 1089.
5 P. Leroy, A. Nicolas and A. Moreau, J. Chromatogr., 282 (1984) 561.
6 T.P. Davis, C.W. Gehrke, C.W. Gehrke, Jr., T.D. Cunningham, K.C. Kuo, K.O. Gerhardt, H.D. Johnson and C.H. Williams, Clin. Chem., 24 (1978) 1317.
7 L.D. Mell, Jr., R.N. Hawkins and R.S. Thompson, J. Liq. Chromatogr., 2 (1979) 1393.
8 N. Seiler, in P.N. Kabra and L.J. Marton (Editors), Clinical Liquid Chromatography, Vol. 2, CRC Press, Boca Raton, FL, 1984, p. 179.
9 R.C. Simpson, H.Y. Mohammed and H. Veening, J. Liq. Chromatogr., 5 (1982) 245.
10 M. Roth, Anal. Chem., 43 (1971) 880.
11 P. Lindroth and K. Mopper, Anal. Chem., 51 (1979) 1667.
12 P.A. Shore, A. Burkhalter and V.H. Cohn, Jr., J. Pharmacol. Exp. Ther., 127 (1959) 182.
13 W. Lorenz, L. Benesch, H. Barth, E. Matejka, R. Mayer, J. Kusche, M. Hutzel and E. Werle, Z. Anal. Chem., 252 (1970) 94.
14 J. Glowinski and L.L. Iversen, J. Neurochem., 13 (1966) 655.
15 J.A. Oates, E. Marsh and A. Sjoerdsma, Clin. Chim. Acta, 7 (1962) 488.

16 J.C. Robert, J. Vatier, B.K. Nguyen Phuoc and S. Bonfils, J. Chromatogr., 273 (1983) 275.
17 C.W. Schubert, R.W. Andrews, W.R. Matson, J. Morrison and E.W. Zink, The Pittsburgh Conference and Exposition on Analytical Chemistry and Applied Spectroscopy, Atlantic City, NJ, March 5—9, 1984, Abstract No. 050.
18 G. Skofitsch, A. Saria, P. Holzer and F. Lembeck, J. Chromatogr., 226 (1981) 53.
19 Y. Tsuruta, K. Kohashi and Y. Ohkura, J. Chromatogr., 146 (1978) 490.
20 J.D.H. Cooper, G. Ogden, J. McIntosh and D.C. Turnell, Anal. Biochem., 142 (1984) 98.
21 A.L. Ronnberg, C. Hansson and R. Hakanson, Anal. Biochem., 139 (1984) 338.
22 Y. Tsuruta, K. Kohashi and Y. Ohkura, J. Chromatogr., 224 (1981) 105.
23 S.J. Lewis, M.R. Fennessy, F.J. Laska and D.A. Taylor, Agents Actions, 10 (1980) 197.
24 N. Subramanian, W. Schinzel, P. Mitznegg and C.-J. Estler, Agents Actions, 8 (1978) 488.
25 L.B. Hough, J.K. Khandelwal and J.P. Green, Brain Res., 291 (1984) 103.