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Analysis of rat brain microdialysate by gas chromatography–high-resolution selected-ion monitoring mass spectrometry

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ABSTRACT

Gas chromatography–high-resolution selected-ion monitoring mass spectrometry was used to analyze catecholamine metabolites in rat brain microdialysate. Dialysate samples were collected in vials containing stable isotope analogues of homovanillic acid (HVA), 3-methoxy-4-hydroxyphenylglycol (MHPG) and 5-hydroxyindoleacetic acid (5HIAA) and analyzed as their trimethylsilyl derivatives. The metabolite levels were monitored at 20-min intervals throughout the time course of the experiment, beginning immediately after surgery and implantation of the dialysis probe and ending 4 h after amphetamine treatment. The levels of HVA were observed to decrease after amphetamine treatment, while those of MHPG and 5HIAA did not change significantly.

INTRODUCTION

High-performance liquid chromatography (HPLC) with electrochemical detection has been extensively utilized for analysis of catecholamines and their metabolites in brain microdialysis of live animals [1–3]. Advantages of the electrochemical detection system are sensitivity and cost. According to the literature, the estimated electrochemical detection limit for catecholamine metabolites is 6 fmol per injection. Mass spectrometric (MS) methods have been used for analyzing catecholamines with similar detection limits in various matrices [4–8] and recently, a low-resolution chemical ionization selected-ion monitoring (SIM) MS method was used to analyze brain microdialysate from rats treated with amphetamine [9]. The purpose of this paper is to evaluate a high-resolution electron ionization gas chromatographic (GC)–SIM-MS method for the analysis of catecholamine metabolites in microdialysate of rat brain and to demonstrate its potential use in biochemical research.

EXPERIMENTAL

Animals

Male Sprague–Dawley rats (190–210 g) were anesthetized with sodium pento-

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barbital and placed in a stereotaxic instrument for surgical implantation of the dialysis probe into the nucleus accumbens region of the brain. The first five samples (Figs. 2–4) were collected immediately after completion of the surgery while the animal was still sedated. The rat was then transferred to a cage where it was allowed to recover from the effects of anesthesia. Beginning approximately 18 h later, dialysate was collected while the animal moved freely within the cage.

Microdialysis matrix

An artificial cerobrospinal fluid (CSF) solution, which had the following composition, was used for the dialysis infusion medium: 120 m*M* NaCl, 3 m*M* KCl, 1 m*M* $MgCl_2$, 24 m*M* $NaHCO_3$, 1 m*M* Na_2HPO_4, 1.8 m*M* $CaCl_2$ and adjusted to pH 7.4 with 1 *M* HCl.

Dialysis probe and pump

The dialysis probe was constructed from a guide cannula and cellulose fiber membrane (MW cut-off 6000; O.D. 250 μm) in a design similar to that of Robinson and Whishaw [10]. The artificial CSF solution was infused at a rate of 1 μl min^{-1} using a Harvard 22 pump from Harvard Apparatus (South Natick, MA, U.S.A.). The samples were collected at 20-min intervals.

Internal standards

Stable isotope standards of [$^{13}C_6$]homovanillic acid ([^{13}C]HVA) and [$^{13}C_6$]3-methoxy-4-hydroxy phenylglycol ([^{13}C]MHPG), both labeled on the six phenyl carbons, were purchased from Cambridge Isotope Labs. (Woburn, MA, U.S.A.). Deuterium-labeled 5-hydroxyindoleacetic acid (5HIAA-d_2) (α-carbon) was purchased from Merck Isotope (St. Louis, MO, U.S.A.). Internal standards were dissolved in ethyl acetate and kept at −25°C. Approximately 40 pmol of each internal standard were used for the direct evaporation method and approximately 80 pmol for the ethyl acetate extraction of samples.

Direct evaporation of rat brain microdialysate

Microdialysate samples (20 μl) were collected at 0°C in a 1-ml Pierce Reactivial® (Rockford, IL, U.S.A.) which contained 40 pmol of each internal standard, 3 μl of 10 m*M* EDTA, and 5 μl of 1 *M* HCl. Drying the samples was facilitated by the addition of 60 μl of acetonitrile to the vial and then evaporating to dryness under a stream of nitrogen at 40°C. The samples were evaporated completely in 10 min.

Solvent extraction of microdialysate

Rat brain microdialysate samples (20 μl) were collected at 0°C in a 1-ml Reactivial which contained 80 pmol of each internal standard, 120 μl of 1 *M* sodium acetate buffer (pH 6.0, 10 m*M* EDTA) and 22 μl of 8 *M* HCl. To the collected microdialysates, 500 μl of ethyl acetate were added, vortex-mixed and centrifuged

(5 min at 200 *g*). The ethyl acetate layer was transferred to another vial and the solvent was removed with a stream of nitrogen at 40°C.

Derivatization

To the dried microdialysate residues, 50 μl of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA)–acetonitrile–dichloromethane (2:1:0.1) were added and heated at 100°C for 30 min.

GC–MS conditions

A VG 70S mass spectrometer was directly coupled with a Hewlett Packard 5890 gas chromatograph. A DB-5 capillary column (25 m × 0.32 mm I.D., 0.25 μm thickness) and an on-column injector purchased from J&W Scientific (Folsom, CA, U.S.A.) were used. Approximately 1 μl from the total volume of 50 μl was injected on column at 115°C, held for 4 min at that temperature and then programmed to 260°C at 12°C min^{-1}. The mass spectrometer was operated at 6000 resolution (10% valley) in the electron ionization mode. For the detection of HVA, molecular ions of both HVA and [^{13}C]HVA (*m/z* 326.1370 and 332.1571, respectively) were monitored. For MHPG, $[M-TMSOCH_2\cdot]^+$ ions of both MHPG and [^{13}C]MHPG (*m/z* 297.1343 and 303.1544, respectively) were monitored. For 5HIAA, molecular ions of 5HIAA and 5HIAA-d_2 (*m/z* 407.1768 and 409.1894, respectively) were monitored. The compounds HVA, MHPG and 5HIAA were monitored by a voltage scanning SIM, and each group was selected at the specified time by magnetic field jumping.

RESULTS AND DISCUSSION

Direct evaporation of microdialysate (approximately 40 μl), followed by derivatization and MS analysis of analytes, at first appeared to be feasible. However, direct evaporation of artificial CSF sample containing standards as well as brain microdialysates did not produce consistent results, especially with MHPG and 5HIAA. The addition of 30 μmol of EDTA to chelate divalent cations did not produce any significant improvement. HVA was the most stable compound of the three metabolites and yielded results which are pharmacologically meaningful. Fig. 1 represents a time course change of HVA concentration in the brain dialysate of a rat treated with amphetamine (2.5 mg kg^{-1}, intraperitoneally).

While the direct evaporation of this volume was not a reliable method of sample preparation, the same technique applied to smaller volumes of approximately 1–2 μl might be quite valid. A lower infusion rate such as 0.1 μl min^{-1}, which would not only decrease the total volume of dialysate to approximately 1 or 2 μl but would increase the concentration of analytes diffused across the membrane, has not been tried.

The results of the solvent extraction method were significantly more reproducible than the direct evaporation technique at the 20 μl (20-min collection) level.

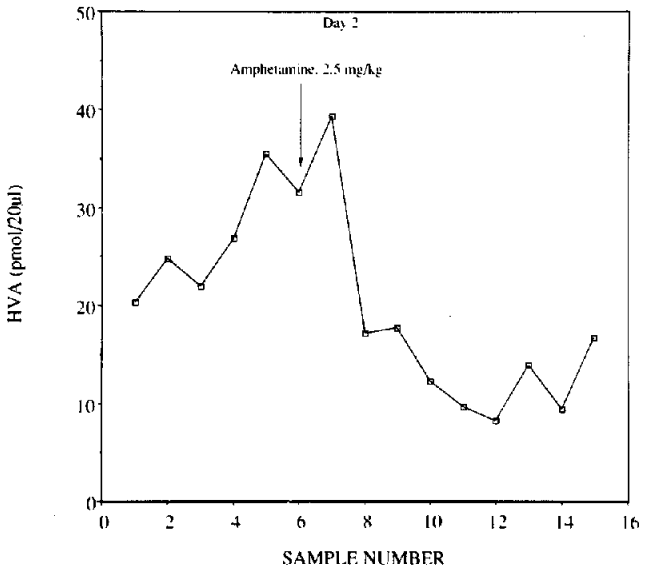

Fig. 1. HVA levels in brain microdialysate from rat treated with amphetamine. Rat was treated with amphetamine (2.5 mg kg^{-1}, intraperitoneally) one day after surgery as indicated in the figure. Microdialysate samples were collected every 20 min one day after surgery and analyzed for HVA by the direct evaporation method (see Experimental).

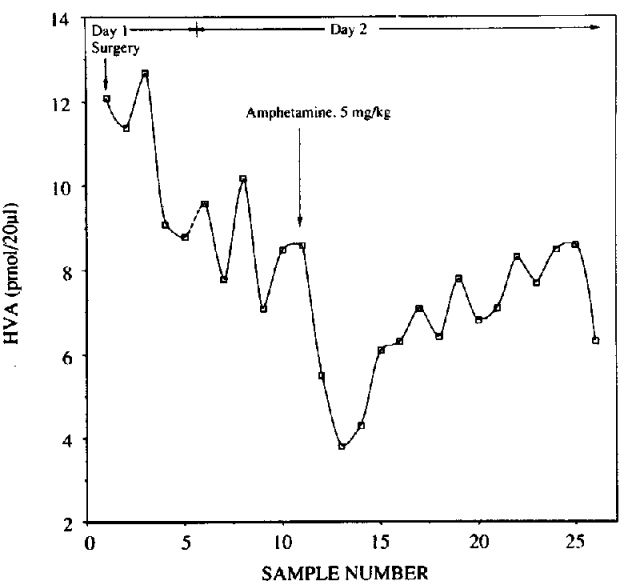

Fig. 2. HVA levels in brain microdialysate from rat treated with amphetamine. Rat was treated with amphetamine (5 mg kg^{-1}, intraperitoneally) one day after surgery as indicated in the figure. Five samples were collected immediately after surgery. Collection was resumed beginning 18 h later. HVA levels were analyzed by the solvent extraction method as described in the Experimental section.

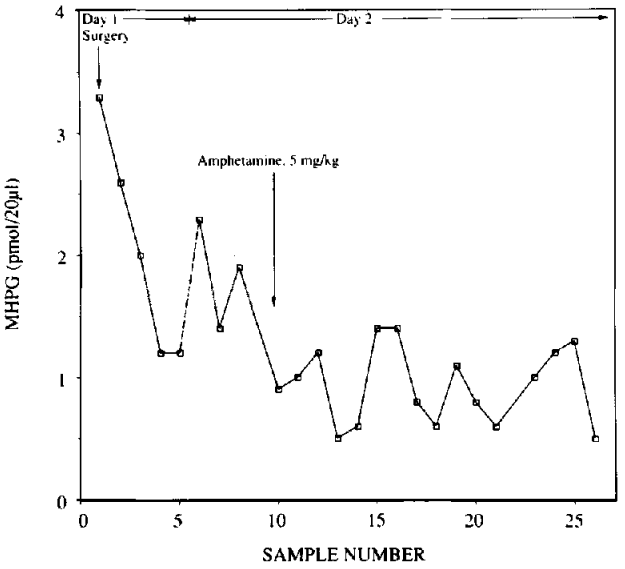

Fig. 3. MHPG levels in brain microdialysate of rat treated with amphetamine. Sample collection conditions are the same as in Fig. 2. MHPG levels were analyzed by the solvent extraction method as described in the Experimental section.

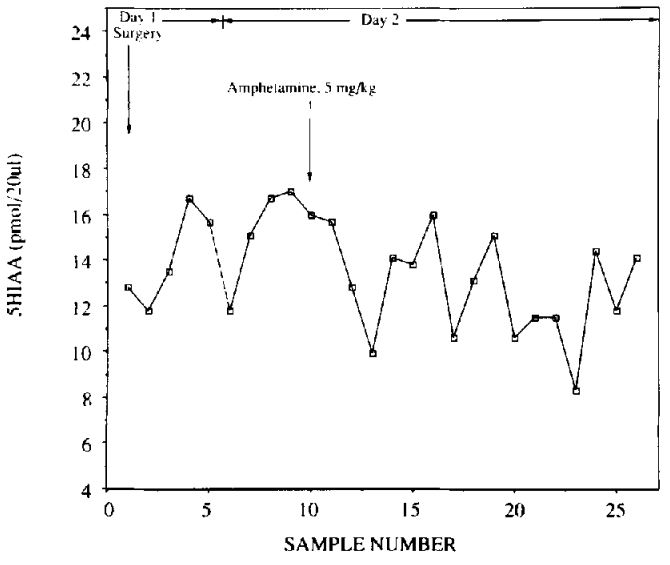

Fig. 4. 5HIAA levels in brain microdialysate of rat treated with amphetamine. Sample collection conditions are the same as in Fig. 2. 5HIAA levels were analyzed by the solvent extraction method as described in the Experimental section.

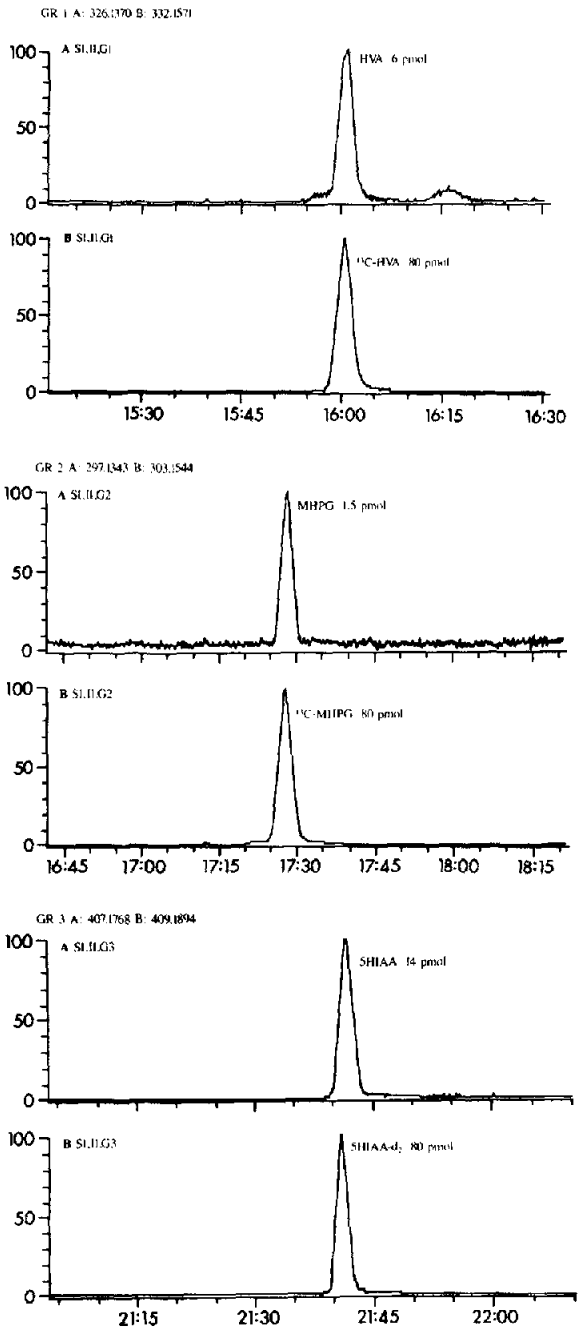

Fig. 5. SIM profiles of rat brain microdialysate: (1) HVA (*m/z* 326.1370), (2) MHPG (*m/z* 297.1343) and (3) 5HIAA (*m/z* 407.1768) and their internal standards monitored from brain microdialysate of rat treated with amphetamine (5 mg kg^{-1}, intraperitoneally). This sample was taken 1.7 h after amphetamine injection.

The levels of three metabolites, HVA, MHPG and 5HIAA, in brain microdialysates of a rat treated with amphetamine (5.0 mg kg^{-1}, intraperitoneally) are presented in Figs. 2–4, respectively. Fig. 5 shows SIM profiles for the levels of HVA, MHPG and 5HIAA and their respective internal standards in 20-μl collected fractions of rat brain microdialysate. The concentration of metabolites in each fraction ranged from 3 to 30 pmol for HVA, 3 to 20 pmol for MHPG and 4 to 25 pmol for 5HIAA depending on the individual animal and sample time points. The sensitivity of MS detection was greater than ten times above signal-to-noise level when 6 fmol of each metabolite were injected. In order to quantitate individual metabolite levels, standard solutions containing 2–50 pmol of the metabolites together with 80 pmol of stable isotope analogues were used to give a linear concentration curve.

There are many factors to be considered when utilizing MS in conjunction with live animal microdialysis techniques for pharmacological studies. Such analytically important factors as membrane type or rate of dialysis have not been examined to date. The purpose of this work was to evaluate high-resolution electron ionization SIM with GC–MS to analyze off-line microdialysis samples. Since the total aqueous volume produced by the small animal brain microdialysate is less than 40 μl in 20 min, the off-line procedure would seem to give greater flexibility than an on-line process such as continuous-flow fast atom bombardment (FAB) for analyzing different analytes in brain microdialysis matrice. Lin *et al.* [11] utilized continuous-flow FAB-MS with HPLC to evaluate the use of MS and microdialysis for pharmacokinetic studies of antibiotics in the rabbit.

Compounds such as catecholamines and their metabolites are sensitive to oxidation–reduction and to the pH of the matrix. Therefore, it is important to spend the minimum amount of time on sample preparation in order to partition target compounds into a more inert environment. The one-step extraction method with ethyl acetate gave a fast partition of metabolites into an organic layer which was then evaporated to dryness within 5 min. Once converted to the trimethylsilyl (TMS) derivatives, the metabolites were stable for at least two weeks at room temperature. Wood and co-workers [4,9] analyzed catecholamines and metabolites as the pentafluoropropionyl derivatives using chemical ionization SIM-MS. In our experience, pentafluoropropionyl derivatives of catecholamines and their metabolites in an ethyl acetate solution as described in the literature [4,9] were stable for only a few hours.

CONCLUSIONS

While it is not our intention to present extensive pharmacological data, any preliminary data produced with this MS method should not only provide a basis for future applications, but also be explainable on a pharmacological basis. We observed a decrease in HVA levels (Figs. 1 and 2) shortly after amphetamine injections. These observations are in agreement with published results [1,2]. The

decreased level of HVA is most likely due to the inhibition of monoamine oxidase activity and the release of the parent neurotransmitters from intraneuronal stores by amphetamine. We did not observe any significant changes in MHPG or 5HIAA levels.

We have applied high-resolution electron ionization GC–SIM-MS to the analysis of catecholamine metabolites in brain microdialysate of rats treated with amphetamine. Although we found that the direct evaporation method was not successful, it still needs to be explored with a lower (0.1 μl min^{-1}) dialysis rate. Off-line analysis of microdialysate with a simple solvent extraction and derivatization method could be extended to other biologically important compounds such as aspartate and glutamate.

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