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# MEASUREMENT OF TYRAMINE IN HUMAN PLASMA, UTILISING ION-PAIR EXTRACTION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH AMPEROMETRIC DETECTION*

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## SUMMARY

An assay for plasma tyramine has been developed which uses ion-pair extraction, reversed-phase ion-pair high-performance liquid chromatography and amperometric detection. Tritiated tyramine is used as the internal standard. The method can measure down to 0.5 ng/ml of tyramine in 1 ml of human plasma and is thus suitable for monoamine oxidase inhibitor studies involving oral dosing with tyramine.

## INTRODUCTION

Tyramine (4-hydroxyphenethylamine) is an indirectly acting sympathomimetic amine found in cheese, fermented foods and red wine [1]. Ingested tyramine is largely inactivated by metabolism to *p*-hydroxyphenylacetic acid catalysed by monoamine oxidase (MAO) enzymes located in the gut, liver and sympathetic nerves [2].

The pressor action of tyramine was found to be dramatically potentiated in some patients, who took cheese whilst receiving MAO inhibitors (MAOI) [3]. This "cheese-reaction" has restricted the use of these drugs in the treatment of depression.

Measurement of plasma tyramine, thus, has an important part to play in the development of safer anti-depressant drugs [4].

Previously, tyramine has been estimated in urine by paper and thin-layer chromatography [5—7] and more recently by high-performance liquid chromatography (HPLC), coupled with fluorimetric [8] or electrochemical detection [9].

In developing a method for the measurement of plasma tyramine, use was

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made of sodium tetraphenylboron [10] to form tetraphenylborate—amine ion-pairs and so enable the extraction of tyramine (at basic pH) into diethyl ether, followed by dissociation of the ion-pair and back extraction into a small volume of acid [11].

Reversed-phase ion-pair HPLC using phosphate-buffered trichloroacetate at pH 5.0 [12] was chosen, so that the retention of tyramine could be controlled by altering the concentration of trichloroacetate (a weak ion-pairing agent) or methanol, depending on the condition of the column. A further degree of selectivity was achieved by use of an amperometric detector to monitor the column effluent as it passed over a glassy carbon thin-layer electrode.

## EXPERIMENTAL

### *Apparatus*

An Altex Model 100A solvent delivery pump was used, fitted with an Altex Model 210 valve (Altex Scientific, Berkeley, CA, U.S.A.) and a 100-$\mu$l loop. A Shandon Hypersil ODS column (5 $\mu$m particle size, 250 × 4.6 mm I.D.) protected by a Co:Pell ODS guard column (25—37 $\mu$m particle size, 50 × 2.1 mm I.D.) (HPLC Technology, Cheshire, U.K.) was employed for chromatography. The column effluent was monitored by a BAS LC-4 amperometric detector equipped with a TL-5 glassy carbon thin-layer cell (Bioanalytical Systems, West Lafayette, IN, U.S.A.). Electrochemical detection was performed at 0.90 V vs. Ag/AgCl reference electrode and chromatograms were displayed on Servoscribe Model RE 541.20 chart recorders (Smiths Industries, London, U.K.). Liquid scintillation counting was performed on a Beckman LS 2800 Scintillation Spectrometer.

### *Reagents*

The internal standard (ring $^3$H) tyramine hydrochloride (specific activity 27 Ci/mmol, 99% pure by paper chromatography, NET-132) was obtained from New England Nuclear (Boston, MA, U.S.A.) and stored at 5°C. The 'cold' tyramine used as primary standard was obtained from Sigma (Poole, U.K.) and sodium tetraphenylboron was purchased from Aldrich (Gillingham, U.K.). Helium and oxygen-free nitrogen were from BOC Special Gases (London, U.K.). All other chemicals were from BDH (Enfield, U.K.).

### *Method*

The method consists of six principal steps: a diethyl ether wash, formation of tetraphenylborate—amine ion-pairs at basic pH, extraction of the ion-pairs into diethyl ether, back extraction into acid, HPLC analysis and fraction collection with scintillation counting.

To 1-ml samples of human plasma add 50 $\mu$l of a 1:1000 dilution of the [$^3$H]tyramine internal standard followed by 10 ml diethyl ether. Mix the tubes in a Multivortex shaker (Denley, Bilinghurst, U.K.) for 10 min, aspirate and discard the upper ether layer. Add 0.3 ml of a solution of sodium tetraphenylboron (5 mg/ml in 1 *M* borate, pH 8.0) and 10 ml diethyl ether. Multivortex the tubes for 10 min. After centrifugation (2050 *g*, 5 min) the tubes are frozen in a mixture of solid carbon dioxide and methylated spirits, and the

ether layer tipped into a fresh set of tubes containing 0.2 ml of 0.07 *M* orthophosphoric acid. Multivortex the tubes for 10 min. After centrifugation (2050 *g*, 5 min) the tubes are again frozen but this time the ether layer is discarded and the tubes blown free of diethyl ether under oxygen-free nitrogen. At this stage the tubes can be stored at −20° C overnight if desired, otherwise add 25 μl of 0.56 *M* potassium hydroxide solution and mix briefly, followed by 150 μl of mobile phase and further mixing.

*Chromatographic conditions*

The mobile phase consists of 0.04 *M* potassium dihydrogen phosphate, 0.03 *M* trichloroacetic acid and 2.5 m*M* disodium EDTA adjusted to pH 5.0. This mixture is filtered through Whatman Gf/f paper and 10% methanol added. The mobile phase is finally helium-degassed prior to pumping at between 1.0 and 1.5 ml/min. At a flow-rate of 1.5 ml/min typical back pressures are 11.03—13.79 MPa. The amperometric detector was set at 0.90 V vs. Ag/AgCl, 1 nA/V, filter C, and three chart recorders connected in series across the signal output to give simultaneous records at three sensitivities: 1 V full scale (1 nA full scale), 2 V full scale (2 nA full scale), and 5 V full scale (5 nA full scale). The column temperature was ambient and in all analytical work constant volumes of 100 μl were injected onto the column by loop over-fill technique. After each injection the column effluent corresponding to the tyramine peak was collected and mixed with scintillation cocktail (Instagel, Packard, U.S.A.) prior to scintillation counting.

*Quantitation*

A stock standard of 10 μg/ml of tyramine was prepared in 0.1 *M* hydrochloric acid and stored at 5° C. On the day of assay this was diluted with 0.01 *M* hydrochloric acid and then used to spike drug-free plasma and prepare a calibration curve over the range 0—200 ng/ml. An internal standard of [$^3$H]-tyramine was chosen to allow for variable recovery of cold tyramine through the extraction and chromatography. Quantitation was achieved by measurement of the peak height cold tyramine/recovered counts [$^3$H]tyramine ratio, for a range of cold tyramine concentrations.

*Plasma samples*

Blood samples were obtained from an indwelling intravenous canula in a forearm vein. They were taken into lithium heparin tubes and the plasma spun down in a refrigerated centrifuge, followed by freezing at −20° C until assayed.

*Dosing*

The bioavailability of oral tyramine was assessed using an 80—800 mg dose, either alone or in combination with an MAO-A inhibitor (Cimoxatone, Delalande, Rueill-Malmaison, France), or an MAO-B inhibitor (MDL 72145, Merrell-Dow, Strasbourg, France). Subjects took no food, drink or drugs that might have contained sympathomimetics during the study periods.

## RESULTS AND DISCUSSION

### *Chromatography*

Asmus and Freed [12, 13] showed that simple acids can replace alkyl sulphates or sulphonates as ion-pairing agents for the separation of the catecholamines and their metabolites. Following their example, we found that the retention of tyramine ($pKa_1$ = 9.5, $pKa_2$ = 10) on reversed-phase HPLC materials was improved by the use of trichloroacetic acid as the ion-pair. Utilising 0.03 *M* trichloroacetic acid buffered to pH 5.0 with 0.04 *M* potassium dihydrogen phosphate and 10% methanol, tyramine had a retention time of 11 min on a Hypersil ODS ($C_{18}$) column, and was well separated from the few other peaks that remained after the extraction. The large peak seen after tyramine in chromatograms of extracted plasma samples (retention time 17.2 min) was also present in tyramine-free aqueous extracts and so is presumed to be the tetraphenylborate from the extraction procedure. Typical chromatograms (Fig. 1) illustrate the selectivity of the chromatographic system used. Blank plasma chromatograms showed no interference which corresponded to the retention of tyramine.

### *Amperometric detection*

Mobile phase pH was critical in determining the optimal applied potential. Changing the pH from 5.0 to 4.5 decreased the retention time of tyramine from 11.0 to 9.9 min and decreased the optimum potential from 0.90 V to 0.85 V vs. Ag/AgCl reference electrode. The usual precautions of adding disodium EDTA to the mobile phase, screening the detector by use of a Faraday Cage and helium degassing were observed.

### *Calibration*

The calibration of each assay was achieved by spiking tyramine into plasma obtained from fasting subjects and constructing a plot of peak height/recovered counts ratio as a function of tyramine concentration. Linearity in standard curves of tyramine was established over an extended range of up to 1 μg/ml in plasma.

### *Recovery*

An estimation of the recovery of tyramine was made using a spike of [$^3$H]-tyramine and collecting the column effluent after the tyramine fraction was eluted, followed by counting in scintillation cocktail. This gave an overall recovery of 52% ($n$ = 4). Recovery across the HPLC column was almost quantitative (95%), indicating that most of the loss occurs at the extraction step.

### *Limit of detection*

Measurement of tyramine (following an oral dose of 80—800 mg) in 1-ml plasma samples was possible at a sensitivity of 2 nA full scale at 0.90 V vs. Ag/AgCl reference electrode on the amperometric detector. With such a setting the absolute limit of detection was 0.5 ng/ml at a signal-to-noise ratio of 2.0. It is likely that a more sensitive assay could be developed by increasing the current amplification on the detector, but this would have to be investigated for any possible interferences.

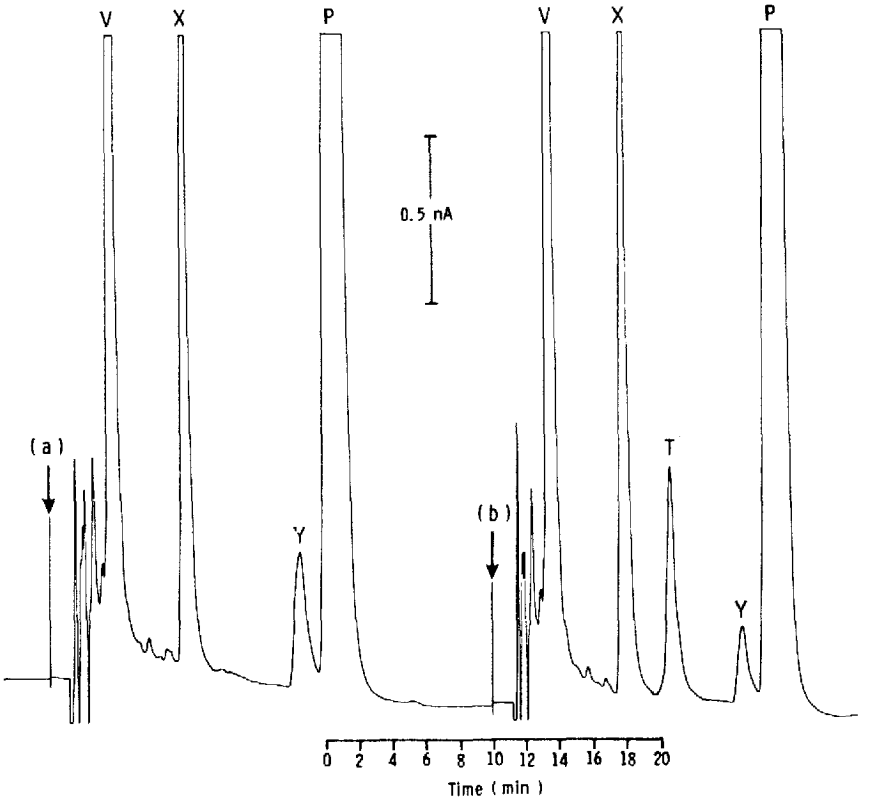


Fig. 1. Chromatograms of (a) blank plasma and (b) plasma taken 30 min after an oral dose of tyramine (400 mg). Concentration of tyramine = 33.5 ng/ml. V = void interference peaks; T = tyramine; P = tetraphenylborate; X and Y = unidentified peaks which do not interfere with the measurement of tyramine. HPLC—amperometric conditions as in text.

*Precision*

*Intra-assay.* Replicate analysis of a pooled plasma sample containing tyramine at a concentration of 22 ng/ml gave a coefficient of variation of 5.3% ($n$ = 10, $\overline{X}$ = 21.95 ng/ml, S.D. = 1.1655).

*Inter-assay.* Assay of the same pooled plasma (stored in portions at −20°C between assays and once thawed not reused) over a two-week period yielded a coefficient of variation of 9.9% ($n$ = 5, $\overline{X}$ = 21.70 ng/ml, S.D. = 2.1389).

*Application*

The method developed for the amperometric determination of tyramine in plasma, provides a means of investigating the sensitivity of MAOI-treated patients to oral tyramine. Such studies are underway in this department and already there have been some interesting results [4, 14]. The mean plasma tyramine concentrations of two normal volunteers following 400 mg of oral tyramine are given in Table I. In this non-medicated situation, plasma tyramine reached a peak within 30 min of ingestion and rapidly returned to baseline. When the subjects were pretreated with the MAO-B inhibitor MDL 72145 and given either (a) the same 400-mg oral dose or (b) an 800-mg oral dose of tyramine, entirely similar profiles were obtained. (Table II). Thus at a dose of

TABLE I

MEAN PLASMA TYRAMINE AFTER A 400-mg ORAL DOSE

| Time after dose (min) | Mean plasma tyramine (ng/ml) |
|---|---|
| 0 | 3.0 |
| 30 | 33.5 |
| 60 | 19.3 |
| 90 | 5.8 |
| 120 | 3.1 |

TABLE II

MEAN PLASMA TYRAMINE AFTER 400 OR 800 mg ORAL TYRAMINE AND 20 mg MDL 72145

| Time after dose (min) | Mean plasma tyramine (ng/ml) | |
|---|---|---|
| | 400 mg Tyramine | 800 mg Tyramine |
| 0 | 0 | 0 |
| 30 | 54.0 | 41.0 |
| 60 | 33.5 | 33.0 |
| 90 | 11.5 | 31.0 |
| 120 | 2.0 | 24.0 |
| 150 | 0 | 12.0 |
| 180 | 0 | 3.0 |
| 210 | 0 | 0 |

TABLE III

MEAN PLASMA TYRAMINE AFTER AN 80-mg ORAL DOSE AND FOUR DAYS OF TAKING CIMOXATONE (20 mg)

| Time after dose (min) | Mean plasma tyramine (ng/ml) |
|---|---|
| 0 | 2.0 |
| 30 | 15.7 |
| 60 | 15.4 |
| 90 | 6.1 |
| 120 | 4.9 |

20 mg, MDL 72145 is unlikely to significantly potentiate the bioavailability of oral tyramine. In another study, the mean plasma tyramine concentrations were recorded after giving two normal volunteers 80 mg of oral tyramine following four days treatment with 20 mg of the MAO-A inhibitor Cimoxatone [15]. These data, shown in Table III, follow a similar pattern, with the MAO inhibitor making no change in the plasma elimination of ingested tyramine. A more detailed report of these studies including clinical details will be published separately.

## CONCLUSIONS

We have shown that tyramine can be reliably measured, following its tetraphenylboron ion-pair extraction from plasma and reversed-phase chromatography with trichloroacetate buffer combined with direct amperometric detection. It seems likely that after suitable modifications this HPLC—amperometric assay could be successfully applied to other biological samples such as urine and food [8, 16] which contain higher concentrations of tyramine. The method may also replace the HPLC—radioactivity method for tyramine, previously used in assessing the pre-systemic metabolism of tyramine in isolated intestinal loop preparations [17].

The proposed method for the HPLC—amperometric analysis of tyramine in human plasma would therefore seem to be suitable for studies on the bio-availability of ingested tyramine and should have particular relevance in the development of MAOI drugs.

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