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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH AMPEROMETRIC DETERMINATION OF PLASMA TYRAMINE

ROGER C. CAUSON* and MORRIS J. BROWN
Department of Clinical Pharmacology, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 0HS (U.K.)

SUMMARY

A method for the measurement of tyramine in human plasma is described. It is based on tetraphenylboron ion-pair extraction and reversed-phase ion-pairing liquid chromatography with amperometric detection. Tyramine can be reliably measured in the range 5–200 ng/ml with an absolute limit of detection of 0.50 ng/ml at a signal-to-noise ratio of 2.0. Correction for variable recovery is made by using a tritiated tyramine internal standard. This assay is suitable for studies on the bioavailability of ingested tyramine and should thus have a role in the development of safer monoamine oxidase inhibitor drugs.

INTRODUCTION

Tyramine is an indirectly acting sympathomimetic amine found in cheese, fermented foods and red wine[1]. Ingested tyramine is largely inactivated by metabolism to *p*-hydroxyphenyl acetic 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 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-pairing HPLC with 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 the use of an amperometric detector to monitor the column effluent passing over a glassy carbon thin-layer cell.

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EXPERIMENTAL

Reagents

Diethyl ether was purchased from May & Baker (Dagenham, U.K.). The internal standard [ring-^{3}H]tyramine hydrochloride (specific activity 27 Ci/mmol, NET-132) was obtained from New England Nuclear (Boston, MA, U.S.A.) and kept at 5°C.

The cold tyramine used as a primary standard was obtained from Sigma (Poole, U.K.) and sodium tetraphenylboron was purchased from Aldrich (Gillingham, U.K.). The helium and oxygen free nitrogen were from BOC special gases (London, U.K.). All other reagents were from BDH (Enfield, U.K.).

Instrumentation

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-μl loop. A Shandon Hypersil ODS column (5 μm particle size, 250 × 4.6 mm I.D.) protected by a CoPell ODS guard column (25–37 μm particle size, 50 × 2.1 mm I.D.) (HPLC Technology, Cheshire, U.K.) was employed for chromatography. The column effluent was monitored by BAS LC-4 amperometric detector, equipped with a TL-5 glassy carbon thin-layer cell (Bioanalytical Systems, West Lafayette, IN, U.S.A.). Amperometric detection was performed at +0.90 V *vs.* Ag/AgCl reference electrode, and chromatograms were displayed on Servoscribe RE 541.20 chart recorders (Smiths Industries, London, U.K.). Liquid scintillation counting was performed in a Beckman LS 2800 scintillation spectrometer.

Extractions from plasma

Human plasma (1 ml) was pipetted into polythene 15-ml diposable test tubes (Sarstedt, Leicester, U.K.) and 50 μl of a 1:1000 dilution of the [^{3}H]tyramine internal standard was added, followed by 10 ml of diethyl ether. The tubes were swirled on a Multivortex shaker (Denley, Bilinghurst, U.K.) for 10 min and the upper (ether) layer was removed by aspiration. A solution of sodium tetraphenylboron (15 mg/ml in 1 *M* borate pH 8.0) was freshly prepared and 0.3 ml was added to the tubes, followed by 10 ml of diethyl ether. The tubes were again swirled for 10 min, centrifuged (2050 *g*, 5 min) and frozen in a mixture of solid carbon dioxide and "methylated spirits" (methanol–ethanol mixture). The ether layer was tipped into another set of glass tubes (Payne, London, U.K.) containing 0.2 ml of 0.07 *M* orthophosphoric acid. These glass tubes were swirled for 10 min, centrifuged (2050 *g*, 5 min) and frozen. The ether layer was discarded and the tubes were blown free of ether under oxygen-free nitrogen. At this stage the tubes could be stored at −20°C overnight if desired, otherwise 25 μl of 0.56 *M* potassium hydroxide solution was added with mixing, followed by 150 μl of mobile phase and further mixing.

Chromatographic conditions

The mobile phase consisted of 0.04 *M* potassium dihydrogen phosphate, 0.03 *M* trichloroacetic acid and 2.5 m*M* disodium ethylenediaminetetraacetic acid (EDTA) adjusted to pH 5.0. This mixture was filtered through Whatman Gf/f paper, methanol was added to 10% (v/v) and the resulting solution purged with helium gas.

Flow-rates of 1.0–1.5 ml/min were used, and at a flow-rate of 1.5 ml/min typical back pressures were 11.03–13.79 MPa. The amperometric detector was held at +0.90 V *vs.* Ag/AgCl, 1 nA/V, filter C, and the chart recorder at a sensitivity of 2 V full scale. The column was used at ambient temperature and in all analytical work constant volumes of 100 μl were injected onto the columns by loop overfill technique. After each injection the column effluent corresponding to the tyramine peak was collected and mixed with Instagel scintillation cocktail (Packard, U.S.A.) prior to scintillation counting.

Standardisation

A stock standard of 10 μg/ml tyramine was prepared in 0.1 *M* hydrochloric acid and stored at 5°C. On the day of the assay, this was diluted with 0.01 *M* hydrochloric acid and then used to spike drug free plasma and to prepare a standard 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 HPLC. Quantitation was achieved by measurement of the peak height cold tyramine/recovered counts [^{3}H]tyramine ratio, for a range of cold tyramine concentrations.

Human studies

The bioavailability of oral tyramine was assessed using a 400 mg dose, either alone or in combination with a MAO-B inhibitor (MDL 72145, Merrell International). A dose of 400 mg was selected, because it is twice the maximum dose likely to be ingested from a tyramine rich diet. Subjects took no food, drink, or drugs that might have contained sympathomimetics during the study periods. Blood samples were obtained from an indwelling intravenous canula in a forearm vein. They were collected in lithium heparin tubes and the plasma was spun down in a refrigerated centrifuge, followed by freezing at −20°C until assayed.

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 approach, we discovered that the retention of tyramine on reversed-phase materials was improved by the use of trichloracetate as the ion-pairing reagent. Utilising 0.03 *M* trichloroacetate, buffered to pH 5.0 with 0.04 *M* potassium dihydrogenphosphate and 10% (v/v) methanol, tyramine had a retention time of 11 min on the Hypersil ODS column and was well resolved from the few other peaks that remained after the extraction. The large peak after tyramine in the 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 specificity of the chromatographic system used. Chromatograms of plasma from fasting individuals showed no interference which corresponded to the 11-min retention time of tyramine.

The mobile phase pH was critical in determining the optimal applied potential for use with a particular thin-layer transducer. Altering the pH from 5.0 to 4.5 de-

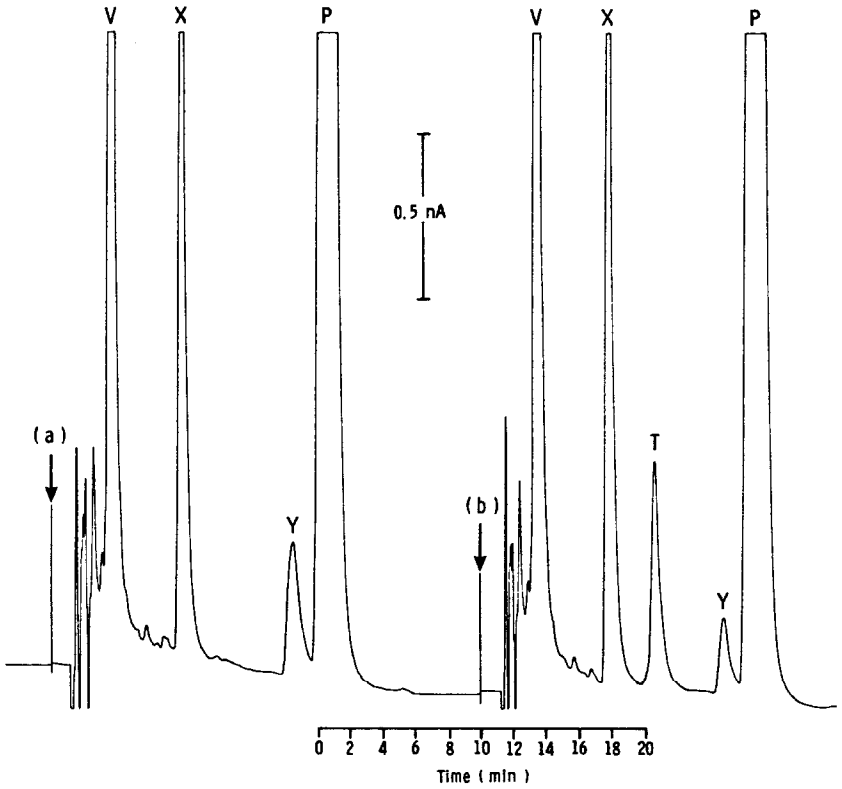

Fig. 1. Chromatograms of (a) plasma obtained after an overnight fast, (b) plasma taken 30 min after an oral dose of 400 mg tyramine. V = Void interference peaks, T = tyramine, P = tetraphenylborate, X, Y = unidentified non-interfering peaks.

creased the retention time of tyramine to 9.9 min and decreased the optimum potential from +0.90 V to +0.85 V *vs.* Ag/AgCl reference electrode. The combination of pH and applied potential chosen was that considered optimal for the measurement of tyramine in human plasma samples. Metal ion and electrical background noise was reduced by inclusion of disodium EDTA in the mobile phase and screening the detector by use of an aluminium Faraday cage.

Recovery

A calculation of the recovery of tyramine was made by using a spike of tritiated tyramine and collecting the HPLC column effluent after the tyramine fraction had been eluted, followed by counting in scintillation cocktail. This gave an overall recovery of 52% (n = 4). Recovery across the HPLC columns was almost quantitative (95%), indicating that the extraction step accounts for most of the loss.

Quantitation

The linearity of the detector response for tyramine was confirmed by spiking plasma obtained from fasting subjects with tyramine and injecting the extracts into the HPLC system. A plot of peak height/recovered counts ratio as a function of

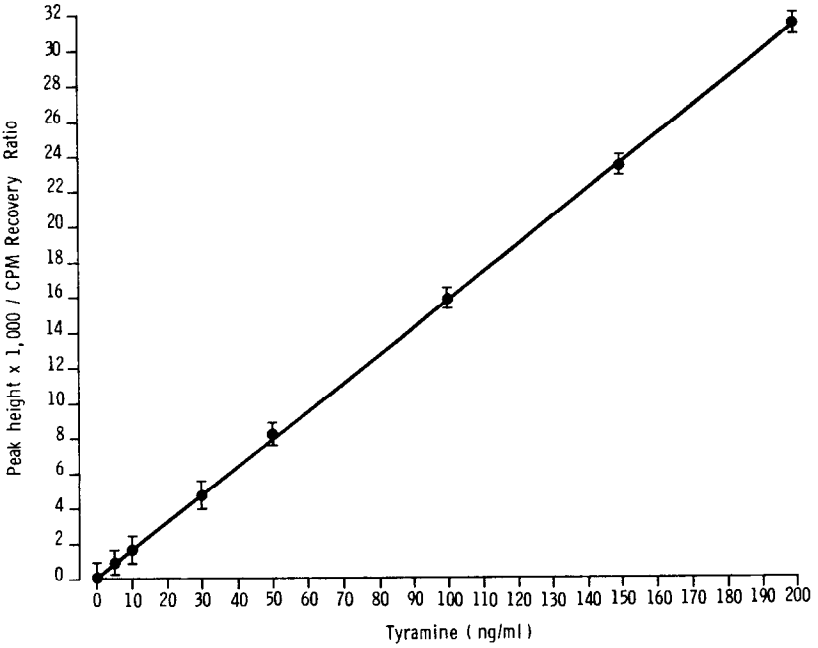

Fig. 2. Mean calibration curve for tyramine in plasma. Each point is the mean ± S. D. of three determinations. (Mean values for 50 ng/ml are: peak height = 43 cm, cpm of tyramine fraction = 5154, giving a ratio of 8.34.).

concentration was then constructed (Fig. 2). Linearity in standard curves of tyramine was established over an extended range of up to 1 μg/ml in plasma. When an applied potential of +0.90 V *vs.* Ag/AgCl and a sensitivity of 2 nA full scale were used the absolute limit of detection for tyramine in plasma was 0.5 ng/ml at a signal-to-noise ratio of 2.0.

Precision

Within-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, $\bar{X}$ = 21.95 ng/ml, S.D. = 1.1655).

Between-assay. Analysis of the same pooled plasma (stored in portions at −20°C between assays and once thawed, not reused) over a 2-week period yielded a coefficient of variation of 9.9% (n = 5, $\bar{X}$ = 21.70 ng/ml, S.D. = 2.1389).

Application

In volunteers, given 400 mg of tyramine by mouth, plasma tyramine reached a peak of 30–50 ng/ml within 30 min of ingestion and rapidly returned to baseline (Fig. 3). A dose of 400 ng was selected, because it is twice the maximum dose likely to be ingested from a tyramine rich diet. Pretreatment of subjects with 20 mg of a new MAO-B inhibitor (MDL 72145, Merrell International) gave entirely similar plasma tyramine profiles, indicating that this drug docs not potentiate the bioavailability of oral tyramine (Fig. 4).

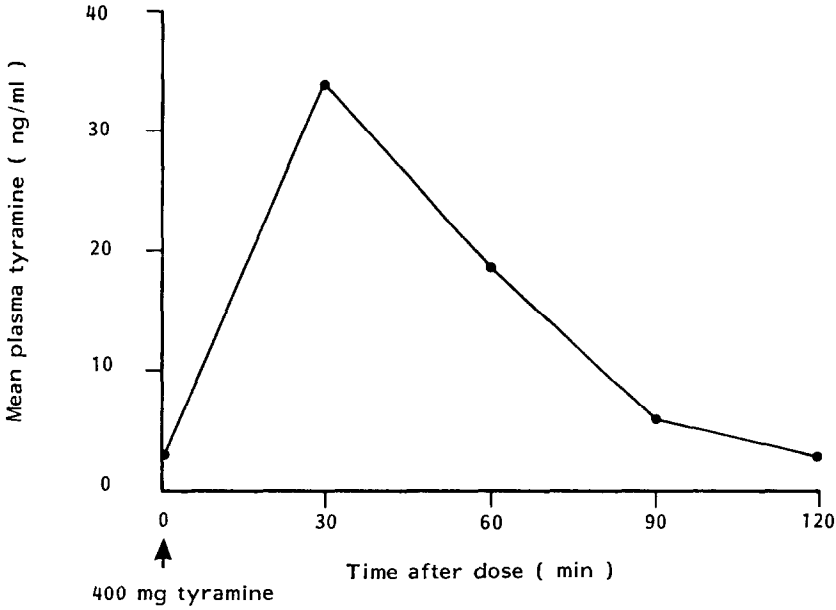

Fig. 3. Mean plasma concentrations of tyramine in volunteers following a single oral dose of 400 mg tyramine.

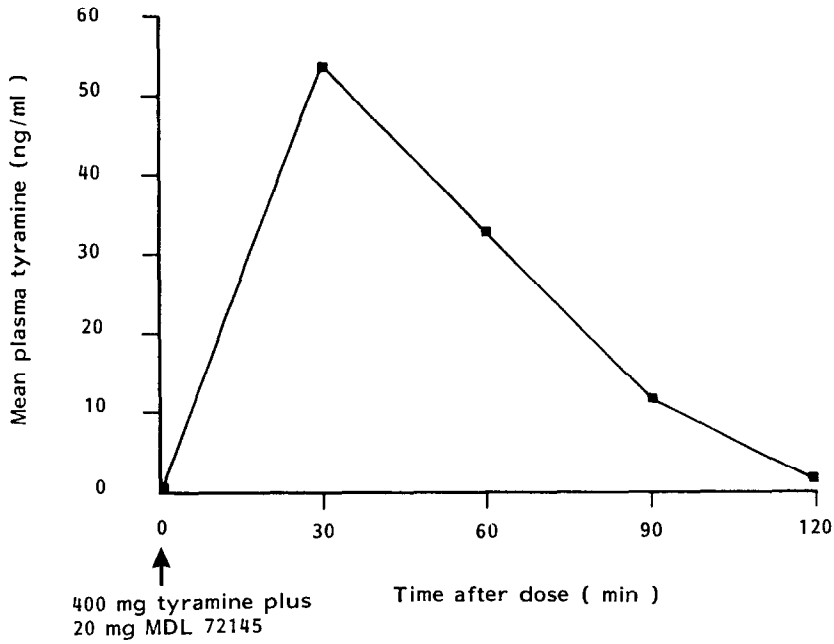

Fig. 4. Mean plasma concentrations of tyramine in volunteers pretreated with 20 mg MDL 72145, following a single oral dose of 400 mg tyramine.

CONCLUSIONS

Plasma tyramine can be reliably measured, following its tetraphenylboron ion-pair extraction, by reversed-phase HPLC with direct amperometric detection. The method is suitable for studies on the bioavailability of ingested tyramine and should have particular relevance in the development of safer MAOI drugs for use in the treatment of depression.

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