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Diurnal changes in plasma levels of 2-pyrrolidinone determined by isotope dilution mass spectrometry

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ABSTRACT

A new capillary gas chromatographic method with mass spectrometric detection for the determination of 2-pyrrolidinone was developed. Using quantification based on stable isotope dilution mass spectrometry by monitoring selected ions in the ammonia chemical ionization mode diurnal changes of 2-pyrrolidinone levels in plasma of three healthy adults were established. Although substantial fluctuations, ranging from 5 to 30 μg/l, occurred during a 24-h daytime period, no consistent picture related to clock time or food intake was obtained. Endogenous oscillations should be taken into consideration, for example when evaluating transdermal penetration of 2-pyrrolidinone, used as enhancer in topical drug formulation.

INTRODUCTION

2-Pyrrolidinone is a normal constituent of human plasma [1,2]. As it is formed from putrescine by rat liver homogenates [3,4], it is considered to originate from polyamine catabolism. Theoretically, it may also originate from the neurotransmitter γ-aminobutyric acid (GABA) by lactamisation. Conversion of 2-pyrrolidinone to GABA in mouse brain [5] and rat tissue [6] endorses a possible relationship between GABA and its lactam. Pharmacotherapeutically, 2-pyrrolidinone is used as a penetration enhancer for active ingredients in topical drug formulations to obtain rapid onset and sustained duration of action [7].

Several methods have been described for the quantification of 2-pyrrolidinone in plasma [1,2] and tissue homogenates [1,2,5]. These include pre-purification by

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solvent extraction [1,2,4] or ion-exchange chromatography [5] and analyses, with [2,5] or without [1,4] previous hydrolysis to GABA, by high-performance liquid chromatography with spectrophotometric [4] or fluorometric [2] detection, and mass fragmentography [1,5].

When studying potential (side) effects of administered 2-pyrrolidinone and the extent and duration of action of 2-pyrrolidinone as a permeation enhancer of the human skin, it is obviously of importance to have knowledge of its plasma concentrations. In such studies endogenous plasma concentrations and notably its possible diurnal variation should be taken into account, as the expected increase during treatment is low and circadian rhythmicities in urinary excretion of polyamine conjugates [8] and metabolites [9] have been reported.

In our hands the previously reported isotope dilution mass fragmentographic method [1] of the N-trimethylsilyl derivative of 2-pyrrolidinone in plasma lacked the required sensitivity to reach plasma levels down to the low μg/l range that were anticipated. We therefore developed a new isotope dilution mass fragmentographic method in which 2-pyrrolidinone is converted in the N-heptafluorobutyryl-isopropyl ester derivative of GABA and analysed by monitoring the $[M+NH_4]^+$ ion in the ammonia chemical ionization mode. The method is applied for the study of diurnal variation in three healthy volunteers.

EXPERIMENTAL

Standards and reagents

2-Pyrrolidinone was obtained from Aldrich Europe (Beerse, Belgium) and 2,2,3,3,4,4-hexadeutero-γ-aminobutyric acid lactam (hexadeuterated 2-pyrrolidinone, 2-pyrrolidinone-d_6) from MSD Isotopes (Montreal, Canada). Heptafluorobutyric anhydride was from Pierce (Rockford, IL, USA) and Carbowax 1000M from Chrompack (Middelburg, The Netherlands). All other reagents were from Merck (Darmstadt, Germany) and were of analytical grade.

Study design

Three apparently healthy volunteers (two males and one female) took part in this study. They underwent extensive pre- and post-study medical checkups and gave their written informed consent. Venous blood samples were drawn in heparinised tubes from each subject at the following times: 08:00, 08:15, 08:30, 09:00, 10:00, 12:00, 14:00, 16:00, 20:00 and 08:00 (next morning). The plasma was isolated by immediate centrifugation at 800 *g*, 4°C for 10 min. The subjects received the same light breakfast (immediately after the first sample was taken at 08:00 h), hot lunch (at 13:00 h) and supper (at 19:00 h). Unlimited consumption of non-alcoholic beverages was permitted during the study. The final blood sample was taken just before breakfast.

Preparation of standard solutions

Stock solutions of 1.25 g/l 2-pyrrolidinone and 2-pyrrolidinone-d_6 in methanol were prepared. Dilutions of the stock solutions in 0.1 mol/l HCl solution resulted in a standard working solution with a final concentration of 62.5 μg/l 2-pyrrolidinone and an internal standard working solution with a final concentration of 125 μg/l 2-pyrrolidinone-d_6, respectively. Histamine was added to the internal standard and standard

working solutions as a carrier [2-pyrrolidinone-d_6/histamine ratio (mol/mol): 1:50; 2-pyrrolidinone/histamine ratio (mol/mol): 1:3.7, respectively].

Isolation and derivatization

To 0.5 ml of plasma were added 25 ng of 2-pyrrolidinone-d_6 (200 μl of the internal standard working solution, see above). After equilibration at room temperature for 30 min, the sample was mixed with 1 ml of sodium borate buffer (50 mmol/l, pH 9.0). The pH was adjusted to 9.0 by adding about three drops of 4 mol/l NaOH. 2-Pyrrolidinone and 2-pyrrolidinone-d_6 were extracted into 7 ml of dichloromethane by gently shaking for 30 min on a GLF shaker (Salm and Kipp, Breukelen, Netherlands) to avoid denaturation of proteins. The dichloromethane (lower) layer was isolated, dried by the addition of a small amount of anhydrous sodium sulphate, and subsequently evaporated to dryness at room temperature under a stream of nitrogen. Since 2-pyrrolidinone is relatively volatile, it is important to discontinue evaporation as soon as the solvent has disappeared. The residue was dissolved in 1 ml of isopropanol–HCl solution (freshly prepared by carefully adding 10 ml of acetylchloride to 100 ml of mechanically stirred distilled isopropanol). Conversion into the isopropyl ester of GABA was performed by heating the tightly capped tubes at 80°C for 8 h. After cooling to room temperature 1 ml of distilled water was added. The mixture was extracted with 6 ml of dichloromethane. A 900-μl aliquot of the aqueous (upper) layer was transferred to a 7-ml teflar-sealable tube and evaporated to dryness at 45°C under a stream of nitrogen. For conversion into the *N*-heptafluorobutyryl-isopropyl ester of GABA, 100 μl each of distilled acetonitrile and heptafluorobutyric anhydride were added. After heating for 30 min at 60°C the solution was evaporated to dryness at room temperature under a stream of nitrogen. The residue was dissolved in 25 μl of ethyl acetate containing 2 g/l Carbowax 1000M. A 4-μl aliquot was analysed by gas chromatography with mass spectrometric detection.

Mass spectrometry

Gas chromatography–mass spectrometry was performed with a Model 5890 gas chromatograph (Hewlett-Packard, Amstelveen, Netherlands) directly coupled to a VG Analytical 70-250 S mass spectrometer (Manchester, UK) and operated under the following conditions: mass spectrometer resolution, $M/\Delta M$, 1000; injector temperature, 250°C; splitless injection mode; helium flow-rate , 0.5 ml/min; oven temperature programme, 80°C, 15°C/min to 140°C, 5°C/min to 160°C, 30°C/min to 250°C; ammonia chemical ionization mode; ion source temperature, 150°C; ionization energy, 150 eV. The column was a 25 m × 0.2 mm I.D. CP Sil-19 CB (film thickness 0.11 μm) fused-silica capillary (Chrompak). The ions at *m/z* 359 and 365, corresponding to the $[M+NH_4]^+$ ions of the *N*-heptabutyryl-isopropyl ester derivatives of GABA and GABA-d_6, respectively, were monitored.

Quantification and quality control

The peak-area ratio of the ions at *m/z* 359 and 365 at the correct retention time was calculated using the VG Analytical 11-250 I data system. Concentrations were computed by means of linear regression analysis using a calibration graph, composed of the corresponding peak-area ratios of various amounts of 2-pyrrolidinone (0–37.5 ng) added to a fixed amount (25 ng) of its deuterated analogue and subjected to the same extraction and derivatisation procedure as described above.

For quality control, we analysed in each series a 0.5-ml aliquot of a pooled serum sample, together with the same aliquot enriched with 12.5 ng of 2-pyrrolidinone (200 μl of the standard working solution, see above).

RESULTS AND DISCUSSION

Methodology and quality control

As, in general, the selectivity of mass fragmentographic assays of low-molecular-weight compounds increases with the selection of ions with increasing m/z, the isolated 2-pyrrolidinone (m/z of the $[M+NH_4]^+$ ion = 103) was subsequently converted into the N-heptafluorobutyryl-isopropyl ester of GABA (m/z of the $[M+NH_4]^+$ ion = 359). Fig. 1 shows the ammonia chemical ionization mass spectra of the *N*-heptafluorobutyryl-isopropyl ester derivatives of GABA (top) and its hexadeuterated analogue (bottom), resulting from their respective 2-pyrrolidinones. The most prominent peaks at m/z 359 and 365, corresponding to their $[M+NH_4]^+$ ions, account for about 30% of the total ion current and proved suitable for selected ion monitoring.

Derivatisation of 2-pyrrolidinone results to some extent in exchange of one or two deuterium atoms for hydrogen atoms. The amount of the hexadeuterated compound was about 89% of the sum of the intensities of the ions at m/z 365, 364 and 363, corresponding to hexa-, penta- and tetradeuterated GABA, respectively. As the purity of the deuterium used in the synthesis of 2-pyrrolidinone-d_6, was 98.5%, the calculated amount of the hexadeuterated compound was 91.3%. In each series the percentage of the hexadeuterated derivative of 2-pyrrolidinone was checked by additional monitoring at m/z 364 (penta) and 363 (tetra) and was shown to be consistent.

Separation between the relatively apolar 2-pyrrolidinone and polar GABA was achieved by selective extraction of the former into dichloromethane at pH 9.0. Addition of GABA up to 3 μg/ml in plasma did not result in increased plasma values for 2-pyrrolidinone with the present method.

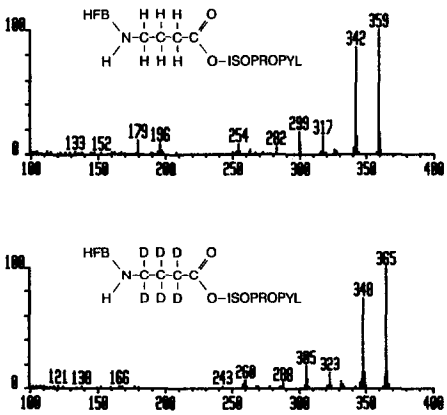

Fig. 1. Ammonia chemical ionization mass spectra of the N-heptafluorobutyryl-isopropyl ester derivatives of GABA and its hexadeuterated analogue, resulting from 2-pyrrolidinone (top) and hexadeuterated 2-pyrrolidinone (bottom), respectively. D = Deuterium.

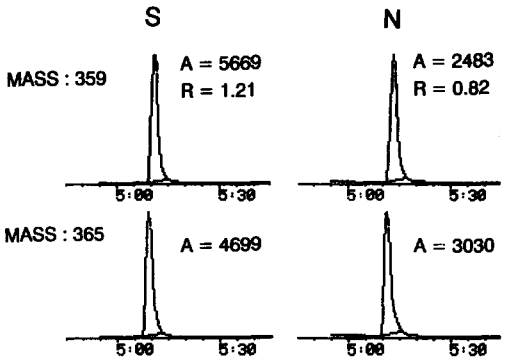

Fig. 2. Mass fragmentograms of the N-heptafluorobutyryl-isopropyl ester derivatives of GABA (*m/z* 359) and its hexadeuterated analogue (*m/z* 365), resulting from 2-pyrrolidinone and hexadeuterated 2-pyrrolidinone, respectively, in a standard (S) containing a mixture of 12.5 ng 2-pyrrolidinone and 25 ng 2-pyrrolidinone-d_6, and a plasma sample of a healthy adult (N) with a concentration of 12.4 μg/l. Time scale in minutes. A, peak area in arbitrary units (counts); R, peak-area ratio of the ions at *m/z* 359 and 365. Time scale in min:s.

Plastic materials may give rise to contamination with 2-pyrrolidinone [1]. Therefore, glass vessels and tubes were used for the storage of all chemicals and standards. All procedures were performed in glassware. However, 2-pyrrolidinone exhibits the peculiar property of adsorption onto silica at neutral and alkaline pH. Therefore, histamine, showing the same property [10], was added to the internal standard solution as a carrier to minimize losses of endogenous 2-pyrrolidinone and its stable isotopically labelled internal standard by this process.

For plasma samples a post-esterification clean-up procedure (*i.e.* extraction with dichloromethane, see Experimental) proved necessary to remove apolar substances that gave rise to interference with naturally occurring 2-pyrrolidinone at *m/z* 359.

Fig. 2 shows mass fragmentograms of 2-pyrrolidinone and its deuterated internal standard for a standard and a plasma sample of a healthy adult.

Table I gives the within- and between-series quality-control data for the endogenous concentration of 2-pyrrolidinone in a pooled serum, together with results of analytical recovery studies. The detection limit for this method (peak/background ratio = 6:1) is about 2 μg/l 2-pyrrolidinone in plasma.

TABLE I

WITHIN- AND BETWEEN-SERIES PRECISION AND RECOVERY FOR DETERMINATION OF 2-PYRROLIDINONE IN POOLED SERUM FROM ADULTS

	n	Concentration (μg/l) (mean ± S.D.)		Recovery (%) (mean ± S.D.)	
Within series	6	18.2	1.1	94.3	4.5
Between series	8	17.4	3.1	102.4	7.3

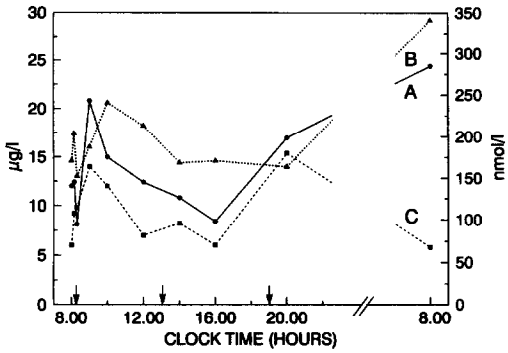

Fig. 3. Diurnal changes in plasma levels of 2-pyrrolidinone, established for two healthy men (A,B) and one woman (C). The arrows indicate the times at which meals were taken.

Diurnal changes

Fig. 3 depicts the results of the study on diurnal changes in plasma levels of 2-pyrrolidinone for three healthy adults. The plasma levels of 2-pyrrolidinone obtained in this study are mostly higher than previously reported for human plasma [1,2]. Although substantial oscillations during a 24 h daytime period occurred, no consistent picture relating to clock time or food intake was obtained.

It is, however, conceivable that fluctuations in the supply of putrescine contribute to fluctuations in circulating levels of 2-pyrrolidinone. Sources of exogenous putrescine are the diet, notably microbially fermented food products [11], and the microbial flora in the colon. The latter produces putrescine, by decarboxylation of ornithine, to counteract a drop in pH caused by carbohydrate fermentation [11,12]. In the small intestine dietary putrescine is likely to become oxidatively deaminated by the locally high activity of diamine oxidase [13], leading to production of Δ'-pyrroline, GABA and possibly 2-pyrrolidinone [14]. Absorption of microbially produced putrescine in the colon, which is low in diamine oxidase activity [13], may increase the levels of circulating putrescine. The latter is, at least partly, degraded to GABA [15], and possibly Δ'-pyrroline and 2-pyrrolidinone. Whether excessive intake of putrescine and consumption of poorly absorbable carbohydrates lead to increases of circulating 2-pyrrolidinone remains to be established.

In conclusion, the average plasma levels of endogenous 2-pyrrolidinone and its diurnal fluctuation should be taken into consideration when evaluating transdermal penetration of 2-pyrrolidinone.

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