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Short communication

Determination of D,L-propargylglycine and N-acetylpropargylglycine in urine and several tissues of D,L-propargylglycine-treated rats using liquid chromatography–mass spectrometry

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Abstract

An experimental animal model with cystathioninuria was obtained by the injection of D,L-propargylglycine into rats. The concentrations of D,L-propargylglycine in urine, several tissues and serum at different times after the injection were measured by liquid chromatography–mass spectrometry. The propargylglycine accumulated rapidly in several tissues and serum of the rats, and reached its maximum level at about 2 h after the injection. Approximately 21.2% of the administered propargylglycine was excreted in urine. N-Acetylpropargylglycine was identified as a new metabolite of propargylglycine in urine. The concentration of propargylglycine was 100 times that of N-acetylpropargylglycine in urine.

1. Introduction

D,L-Propargylglycine has been reported to inactivate rat liver cystathionine γ-lyase and to lead to a rapid decrease in the hepatic activity of the enzyme when administered to mice [1]. It has been reported in previous papers [2,3] that an experimental animal model with cystathioninuria was obtained by injection of D,L-propargylglycine into rats and the concentrations of propargylglycine in urine and several tissues have been determined by isotachophoresis [3]. However, it was impossible to identify N-acetylpropargylglycine (NAc-propargylglycine) in urine using isotachophoresis. Recently, atmospheric pressure ionization mass spectrometry combined with high-performance liquid chromatography (LC–API-MS) has shown promise in various fields [4–7].

Using LC–API-MS, we have determined propargylglycine in urine and several tissues of propargylglycine-treated rats. The results agreed with those obtained by using isotachophoresis [3]. We suggested the presence of acetylated derivatives and identified NAc-propargylglycine using LC–API-MS. The aim of this work was to demonstrate the applicability of this method to

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qualitative and quantitative analyses of biological samples for new metabolites.

2. Experimental

2.1. Reagents

D,L-Propargylglycine and NAc-propargylglycine were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical-reagent grade.

2.2. Animals and urine samples

Male Wistar strain rats (average body mass 200 g) were transferred to individual metabolic cages. Experimental cystathioninuria was induced in the rats by intraperitoneal administration of D,L-propargylglycine (100 mg/kg body mass) as reported previously [8]. Physiological saline solution (0.5 ml) was injected into control rats (three rats). At various post-injection times, urine was collected in a vessel containing 0.5 ml of 2 *M* acetic acid.

2.3. Determination of D,L-propargylglycine and NAc-propargylglycine

Rats were killed by decapitation at various times after the injection of D,L-propargylglycine. Each tissue (1 g) was homogenized in three volumes of 2% sulfosalicylic acid and the homogenate was centrifuged at 2000 *g* for 15 min. The supernatant was applied to a column containing 10 ml of Diaion SK-1 (H^+-form of sulfonated cation exchanger, 100 mesh; Mitsubishi Kasei, Tokyo, Japan), washed with 100 ml of water and eluted with 50 ml of 2 *M* ammonia solution. Each eluate was evaporated to dryness under reduced pressure. The washed aqueous fraction was applied to a column containing 10 ml of Diaion SA-100 ($HCOO^-$-form of anion exchanger, 100 mesh; Mitsubishi Kasei) washed with 30 ml of water and eluted with 50 ml of 10% formic acid. The eluate was dried under reduced pressure. The urine sample was also applied to a column and treated as a supernatant of tissues. Each aliquot of both residues was analysed by LC–API-MS.

2.4. Instrumentation

The apparatus used was a Hitachi L-6200 HPLC instrument, equipped with a 5-μm Inertsil ODS-2 packed column (150 × 4.6 mm I.D.) from Gasukuro Kogyo (Tokyo, Japan), connected to a Hitachi M80B mass spectrometer–computer system, through the API interface [9]. The nebulizer and vaporizer temperatures were 255 and 380°C, respectively. Determinations of synthetic and urinary propargylglycine and NAc-propargylglycine were carried out with a mobile phase composed of 15% acetonitrile in 100 mmol/l ammonium acetate at a flow-rate of 0.9 ml/min, and scanned using a mass spectrometer at a rate of 4 s per scan.

3. Results and discussion

Mass chromatograms and mass spectra of synthetic D,L-propargylglycine and NAc-propargylglycine, obtained using the LC–API-MS system, are shown in Fig. 1. In the LC–API-MS system, the quasi-molecular ions $[M+H]^+$ of D,L-propargylglycine and NAc-propargylglycine were observed as base peaks at *m/z* 114 for D,L-propargylglycine and *m/z* 156 for NAc-propargylglycine. Additional ions, $[M-COCH_3]^+$ and $[M+NH_4]^+$ were also detected. The calibration graphs for different concentrations of D,L-propargylglycine and NAc-propargylglycine were linear over the concentration range 200–1000 ng. The solutions of D,L-propargylglycine and NAc-propargylglycine were treated with ion-exchange resins (Diaion SK-1 and SA-100) as described under Experimental. The 2 *M* ammonia solution eluate from Diaion SK-1 and the 10% formic acid eluate from Diaion SA-100 were analysed using LC–API-MS. D,L-Propargylglycine (*m/z* 114) was detected in the 2 *M* ammonia solution eluate and NAc-propargylglycine (*m/z* 156) was detected in the 10% formic acid eluate, as shown in Fig. 2B and A, respectively. The retention time of D,L-prop-

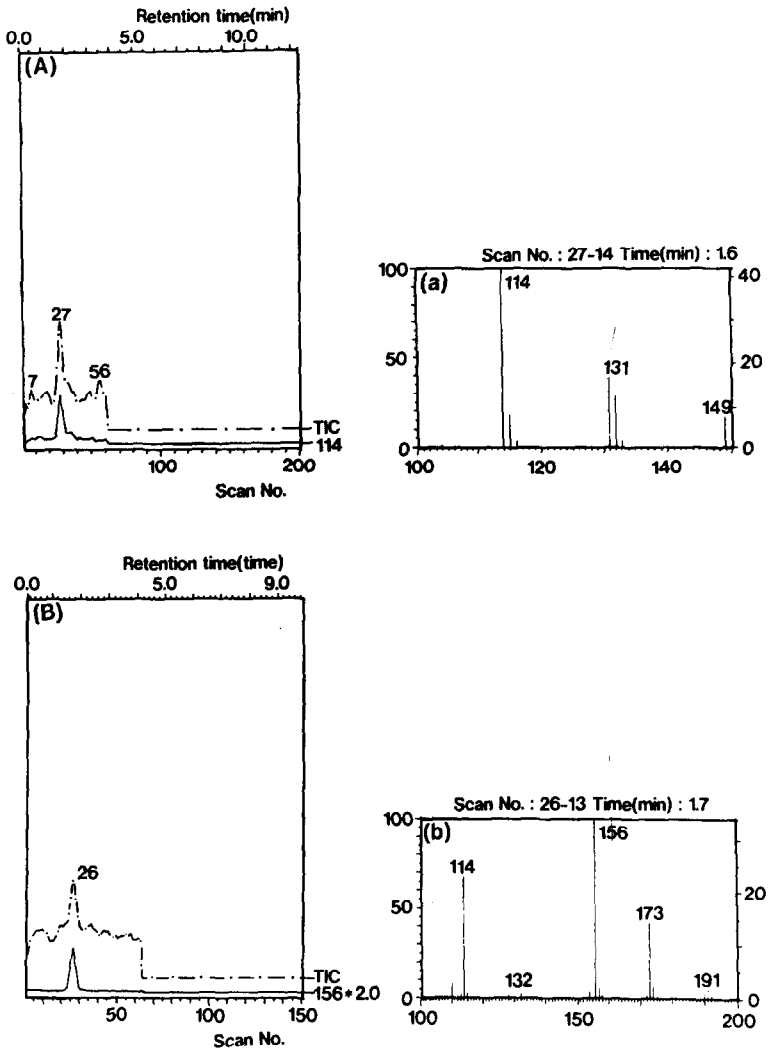

Fig. 1. Mass chromatograms and spectra of D,L-propargylglycine (A, a; *m/z* 114) and NAc-propargylglycine (B, b; *m/z* 156). Mass spectra scanned at the peak tops of each mass chromatogram (A and B). Chromatographic conditions: mobile phase, acetonitrile–100 mmol/l ammonium acetate (15:85); flow-rate, 0.9 ml/min. The mass spectrometer was scanned from *m/z* 100 to 150 or 200 at rate of 4 s per scan.

argylglycine was ca. 1.6 min and that of NAc-propargylglycine was ca. 1.5 min. The recoveries of synthetic propargylglycine and NAc-propargylglycine (500 ng per 10 μl) after treatment with ion-exchange resins were determined five times; they were $90.1 \pm 3.1\%$ (range 86.0–93.4%) and $86.8 \pm 5.6\%$ (range 83.0–96.3%), respectively. The detection limit for both compounds is 50 ng. These results indicate that this method is reliable for the measurement of both substances.

Mass chromatograms of the fraction containing D,L-propargylglycine from urine samples of the rats that had been treated with propargylglycine and not treated are shown in Fig. 3A and B, respectively. Propargylglycine was not detected in the control urine (Fig. 3B), but it was detected in the urine after injection of D,L-

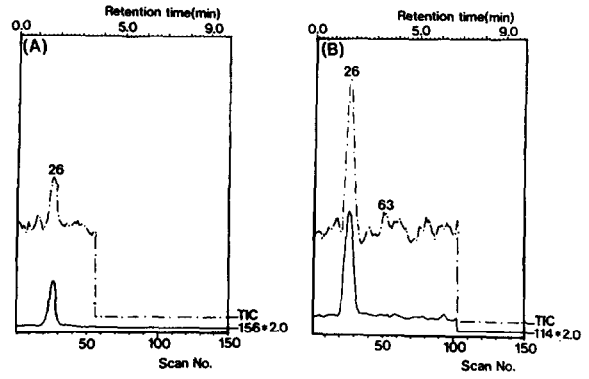

Fig. 2. Mass chromatograms of the eluates with 2 *M* ammonia solution (B) and 10% formic acid (A). A mixture of synthetic D,L-propargylglycine and NAc-propargylglycine was applied to Diaion SK-1, washed with water and eluted with 2 *M* ammonia solution. The 2 *M* ammonia solution eluate was evaporated under reduced pressure. The effluent and aqueous fraction were combined, applied to Diaion SA-100 and washed with 50 ml of 10% formic acid. The eluate was evaporated under reduced pressure. The ammonia solution eluate (B) and formic acid eluate (A) were analysed by LC–API-MS.

propargylglycine, and the retention time was almost the same as that of the standard compound (Fig. 3A). NAc-propargylglycine was not identified in the urine of propargylglycine-treated rats. Therefore the 10% formic acid eluate from the urine of propargylglycine-treated rats was analysed using LC–API-MS. The mass chromatogram and spectrum of this eluate are shown in Fig. 4A and a.

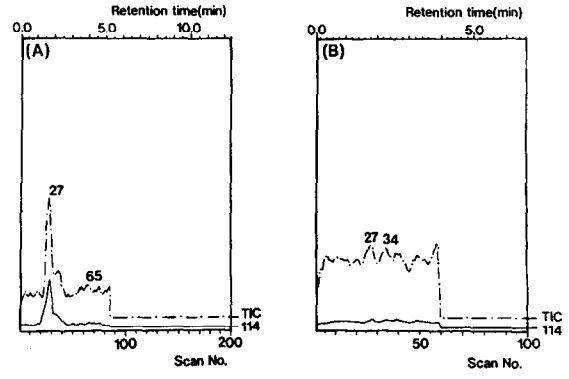

Fig. 3. Mass chromatograms of 2 *M* ammonia solution eluate from urine samples of D,L-propargylglycine-treated rats (A) and normal rats (B). The urine samples were prepared as described under Experimental.

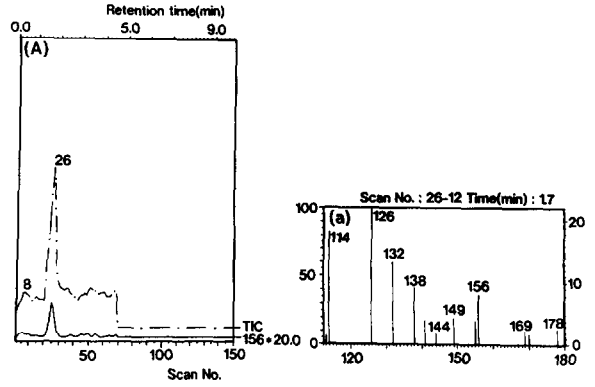

Fig. 4. Mass chromatogram (A) and spectrum (a) of 10% formic acid eluate (Diaion SA-100) from urine of D,L-propargylglycine-treated rats.

The quasi-molecular ion (*m/z* 156) of NAc-propargylglycine was detected on the mass chromatogram. An additional ion $[M - COCH_3]^+$ (*m/z* 114), was also detected. These results indicate that NAc-propargylglycine was identified as a new metabolite of propargylglycine. The contents of propargylglycine and NAc-propargylglycine in the urine at different times after the rats had been injected with D,L-propargylglycine are shown in Fig. 5. These results indicate that NAc-propargylglycine was produced in vivo after injection of D,L-propargylglycine into the rats. The concentration of NAc-propargylglycine was very low; the content

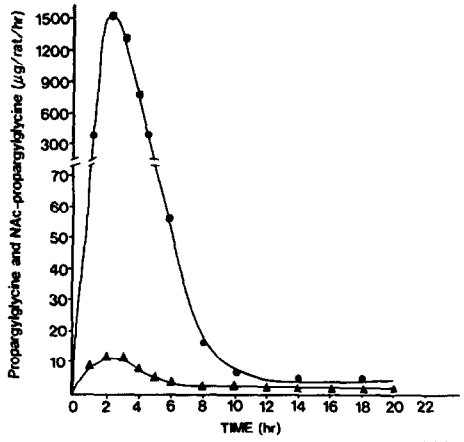

Fig. 5. Contents of (●) D,L-propargylglycine and (▲) NAc-propargylglycine in urine samples at different times after the injection of D,L-propargylglycine.

of propargylglycine was 100 times greater than that of NAc-propargylglycine in the urine.

The concentrations of propargylglycine in various tissues and serum are shown in Fig. 6. The concentration of NAc-propargylglycine in tissues was too low to be detected. The urinary excretion of propargylglycine and NAc-propargylglycine reached their maxima at about 2 h after injection. Apart from in the urine, the content of propargylglycine in the liver, kidney, brain and serum also reached its maximum at about 2 h after injection. The concentrations of D,L-propargylglycine in the liver and serum remained longer than those in urine, kidney and brain. Approximately 21.2% of administered D,L-propargylglycine was excreted into the urine within 6 h and could not be detected in the urine by about 12 h after administration (Fig. 5). These results suggest that D,L-propargylglycine arrived first in the liver and then kidney; part of the D,L-propargylglycine was acetylated in vivo, and was quickly excreted by way of serum into the urine.

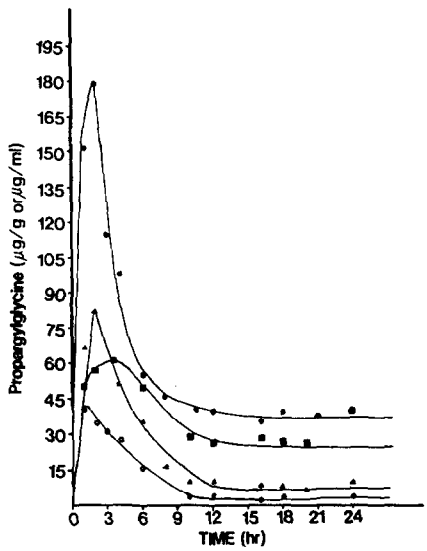

Fig. 6. Contents of D,L-propargylglycine at different times in the liver (●), kidney (▲), serum (■) and brain (○) of D,L-propargylglycine-treated rats.

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