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Determination of hypotaurine and taurine in blood plasma of rats after the administration of L-cysteine.

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Abstract

A method for the simultaneous determination of hypotaurine and taurine was developed. The method consisted of the elimination of urea, which interfered with the determination of hypotaurine, by immobilized urease, and determination of hypotaurine and taurine with an amino acid analyzer. The analyzer equipped with a cation-exchange column was operated at 32 degrees C with 0.2 M sodium citrate buffer, pH 2.8. Using this method, the dynamics of hypotaurine and taurine in blood plasma of rats was studied after the intraperitoneal injection of L-cysteine. The concentration of cysteine reached the maximum 1 h after L-cysteine loading. The concentration of hypotaurine and taurine increased in parallel and reached the maximum 2 h after L-cysteine loading. These changes seem to indicate the precursor-product relationship of these substances and the rapid conversion of hypotaurine to taurine in vivo.

KEYWORDS: hypotaurine, taurine, determination, cysteine metabolisn, amino acid analysis

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— Brief Note —

Determination of Hypotaurine and Taurine in Blood Plasma of Rats after the Administration of L-Cysteine

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A method for the simultaneous determination of hypotaurine and taurine was developed. The method consisted of the elimination of urea, which interfered with the determination of hypotaurine, by immobilized urease, and determination of hypotaurine and taurine with an amino acid analyzer. The analyzer equipped with a cation-exchange column was operated at 32°C with 0.2 M sodium citrate buffer, pH 2.8. Using this method, the dynamics of hypotaurine and taurine in blood plasma of rats was studied after the intraperitoneal injection of L-cysteine. The concentration of cysteine reached the maximum 1 h after L-cysteine loading. The concentration of hypotaurine and taurine increased in parallel and reached the maximum 2 h after L-cysteine loading. These changes seem to indicate the precursor-product relationship of these substances and the rapid conversion of hypotaurine to taurine *in vivo*.

Key words : **hypotaurine, taurine, determination, cysteine metabolism, amino acid analysis**

Taurine (2-aminoethanesulfonic acid) is one of the end products of sulfur-containing amino acids and is contained in high concentration in various mammalian tissues including excitable tissues (1). Taurine may be synthesized in mammals from L-cysteine via cysteinesulfinic acid and hypotaurine (cysteinesulfinic acid pathway) (2), via cysteamine and hypotaurine (cysteamine pathway) (3) or via cysteic acid (4). In the former two pathways, hypotaurine is the immediate precursor of taurine. The formation of hypotaurine from L-cysteine in rats (5) and the conversion of hypotaurine to taurine in rats (6, 7) and mice (7, 8) have been reported. However, the dynamic metabolic profile of hypotaurine *in vivo* has not been fully studied. We have developed a method for the simultaneous determination of hypotaurine and taurine in blood plasma, which consists of the elimination of urea with immobilized urease and the ion-exchange chromatography with an amino acid analyzer. Using this method, the dynamic profile of hypotaurine and taurine in rat blood after L-cysteine loading was investigated.

L-Cysteine (free base), hypotaurine and taurine were purchased from Sigma Chemical Company, St. Louis, MO, USA. Urease-500 Enzygel (immobilized urease) was purchased from Boehringer Mannheim Yamanouchi Company, Tokyo, Japan. Sephadex G-25 (fine) was obtained from Pharmacia-LKB Biotechnology, Uppsala, Sweden. All other reagents used were

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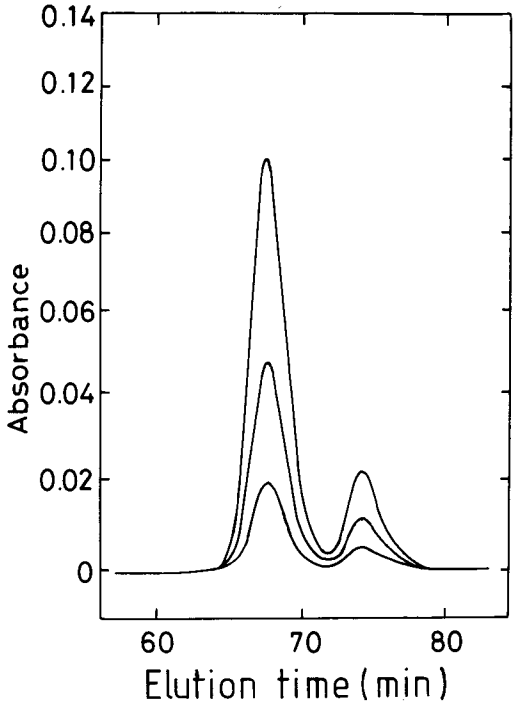

Fig. 1 A profile of the analysis of taurine and hypotaurine in the blood plasma of rats 3h after the intraperitoneal injection of L-cysteine (5 mmol per kg body weight). Upper and middle tracing, absorbance at 570 nm ; lower tracing, at 440 nm. The first peak corresponds with taurine, and the second with hypotaurine.

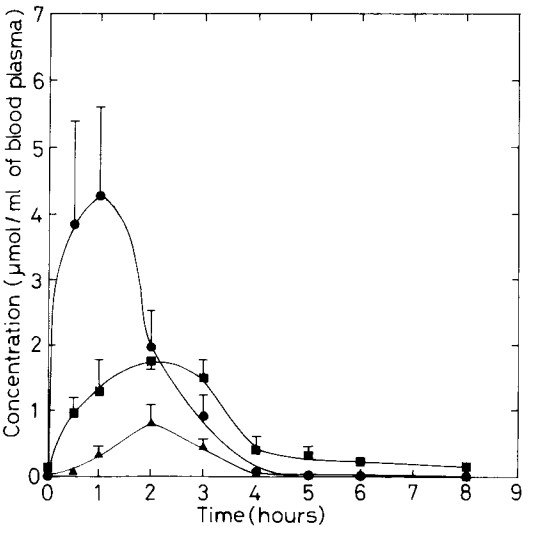

Fig. 2 The concentration of cysteine (●—●), hypotaurine (▲—▲) and taurine (■—■) in the blood plasma of rats after the intraperitoneal administration of L-cysteine (5 mmol per kg of body weight). Values are the mean obtained from three animals. Bar indicates standard deviation.

of analytical grade and purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Male Wistar rats weighing 180-240 g were maintained on a commercial rat diet (MF, Oriental Yeast Company, Ltd., Tokyo, Japan). L-Cysteine solution (0.5 M in water) was freshly prepared and intraperitoneally injected rats at a dose of 5 mmol/kg of body weight.

Blood was withdrawn at the time indicated in Fig. 2 under ether anesthesia from a cervical artery through a polyethylene catheter and collected in a centrifuge tube containing 0.2 ml of heparin solution (100 units/ml). Blood plasma was obtained by centrifugation at 1,200 × *g* for 10 min at 4 °C.

The concentrations of hypotaurine and taurine were determined with a Hitachi KLA-5 amino acid analyzer (Hitachi Co., Ltd., Tokyo, Japan) at 32 °C using a column of Hitachi custom ion-exchanger No. 2614 (0.9 × 55cm) and 0.2 M sodium citrate buffer (pH 2.8) at a flow rate of 0.5 ml/min. Under these conditions, taurine was eluted at 67.5 min and hypotaurine at 74 min, and both peaks were well separated. When deproteinized blood plasma was analyzed, however, urea was eluted at 77 min, and it partially overlapped with the peak of hypotaurine. Therefore, the interference by urea was eliminated as follows.

Blood plasma (1.0–3.6 ml) was applied to a column of Sephadex G-25 (fine, 1.6 × 36cm) at 4 °C, and elution was carried out with water, collecting 3-ml fractions. A peak containing proteins was eluted in fractions 8–15, and a peak containing low molecular weight substances including hypotaurine, taurine and urea was in fractions 16–32. Both peaks were well separated. The fractions of the latter peak were combined and evaporated to dryness under a reduced pressure using a flash-evaporator at 40 °C. The residue was dissolved in 2 ml of water. To this solution, 10 mg of Urease-500 Enzygel (powder) was added, and the mixture was incubated at 35 °C for 30 min while shaking at 100 strokes/min.

Then Urease-500 Enzygel was removed by centrifugation at 1,200 × *g* for 10 min, and aliquots of the resulting supernatant were analyzed with the amino acid analyzer as described above. By this procedure, urea was removed almost completely, and the recoveries of hypotaurine and taurine were 90 ± 4 and 92 ± 3 %, respectively. The spontaneous oxidation of hypotaurine to taurine was not detected when examined in the recovery experiments. A typical chromatogram obtained by the present method is illustrated in Fig. 1. The first peak was eluted at the same elution time as that of taurine, and the second peak was eluted at that of hypotaurine. The identity of the peak of hypotaurine was confirmed by the treatment of the sample with hydrogen peroxide solution. By this treatment, the peak of hypotaurine disappeared completely and that of taurine increased accordingly, indicating that the second peak was hypotaurine, which was oxidized to taurine.

The concentration of cysteine was determined by the method of Gaitonde (9). As shown in Fig. 2, the concentration of cysteine increased immediately after the L-cysteine administration, reached the maximum 1 h, and returned to the control level 5 h after the L-cysteine administration.

Hypotaurine was not detected in blood plasma before the L-cysteine administration, but it appeared after the L-cysteine loading, and its concentration reached the maximum 2 h later. The concentration of hypotaurine returned to the control level 4 h after the L-cysteine administration.

The concentration of taurine increased almost in parallel with that of hypotaurine and reached the maximum at the same time as hypotaurine. The concentration of taurine returned to the control level 8 h after the L-cysteine loading. The patterns of these changes seem to indicate that a precursor-product relationship exists among these substances.

It has not been established whether the oxidation of hypotaurine to taurine is enzymatic (10, 11) or non-enzymatic (12). The accumulation of hypotaurine has been reported in regenerating rat liver, and a mechanism for the conversion of hypotaurine to taurine has been proposed (13). The present results seem to indicate that the conversion of hypotaurine to taurine *in vivo* was rapid and proceeded faster than that of L-cysteine to hypotaurine. The appearance and disappearance of hypotaurine and taurine in the blood plasma reflect complicated processes of uptake, metabolism and release of these compounds by various organs. Further study is needed on the mechanism of hypotaurine oxidation and the participation of organs in these processes.

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