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ISCHEMIC CHANGE OF ORGANIC ACIDS IN KIDNEY

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SUMMARY

Organic acids in rabbit renal tissue biopsy were analyzed by capillary column gas chromatography—mass spectrometry. The change of these organic acids under ischemic conditions was determined over 60 min after clamping the renal artery and vein. The results showed that lactic acid, glycolic acid, 2-hydroxybutyric acid, 3-hydroxypropionic acid, 2-methylglyceric acid, glyceric acid and malic acid increased at 4 and 6 min after clamping, but then decreased at 15 min. Glycerol increased 2 min after clamping and then decreased. However, 3-deoxyaldonic acids of 3-deoxytetronic acid, 3-deoxy-2-C-hydroxymethyltetronic acid and 3-deoxypentonic acid decreased in the renal tissue biopsy from 2 min after clamping.

INTRODUCTION

In a previous study, the authors analyzed organic acids in renal tissue biopsy from patients with renal disease, and identified 4-hydroxybutyric acid and 4-hydroxy-2-butenoic acid in renal tissue for the first time [1]. Recently, the organic acids in various tissue specimens have been analyzed by gas chromatography—mass spectrometry (GC—MS) [2]. Haraguchi et al. [3] analyzed the organic acids in rat heart muscle under ischemic conditions using GC—MS and showed that lactic acid, glycolic acid and 3-deoxyaldonic acids increased until 4 min after decapitation, but then decreased at 6 min after decapitation; 2-deoxytetronic acid and dideoxypentonic acid were markedly increased 6 min after decapitation.

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We have attempted to determine the metabolic change of organic acids in renal tissue under ischemic conditions using GC—MS. Renal ischemia was caused by clamping the renal artery and vein, a method often used as an experimental ischemic acute renal failure model [4].

MATERIALS AND METHODS

Sample preparation

Biopsied samples of the right kidney were obtained from five male rabbits (2—2.5 kg) before and after clamping the renal artery and vein. Renal biopsy samples were obtained at 2, 4, 6, 10, 15, 30, and 60 min after clamping to examine the time-related change of organic acids. The biopsy samples obtained were immediately frozen in dry ice—acetone. Renal tissue (5 mg wet weight) was removed from each specimen, and homogenized in 0.2 ml of cold saline. After the addition of 0.3 ml of cold saline and 50 μg of *p*-(*n*-amyl)benzoic acid (Tokyo Kasei Co., Tokyo, Japan) as an internal standard, the homogenized solution was deproteinized with 3 ml of cold ethanol, and centrifuged at 25,000 *g* for 10 min. The precipitate was washed with cold ethanol and centrifuged again. The collected supernatant was concentrated to 0.5 ml under a stream of nitrogen, and 0.5 ml of distilled water was added to the concentrate. The solution was acidified to pH 1 with 1 *N* hydrochloric acid and extracted thrice with 3 ml of ethyl acetate. The organic phase was dehydrated over anhydrous sodium sulfate and dried under a nitrogen stream. The keto groups were methoximed with 1 mg of methoxylamine hydrochloride at 60°C for 30 min. After evaporation under a nitrogen stream, the organic acids were trimethylsilylated with 40 μl of bis-(trimethylsilyl)-trifluoroacetamide (Pierce, Rockford, IL, U.S.A.) and 10 μl of trimethylchlorosilane (Pierce) at 60°C for 1 h. Aliquots of the samples were subjected to GC and GC—MS analysis.

Gas chromatography and gas chromatography—mass spectrometry

For quantitation of the compounds, a Shimadzu GC-6A gas chromatograph equipped with an OV-101 open tubular glass capillary column (30 m × 0.25 mm I.D.) was used. The column temperature was programmed from 70°C to 260°C at 3°C/min. Peak areas and retention times were determined with an on-line Shimadzu Chromatopac C-RIA computer.

For identification of the compounds, a JEOL JMS D-300 mass spectrometer with a Hewlett-Packard 5710 A gas chromatograph and a JMA 2000 data processing system were used. Mass spectra were recorded at an ionizing voltage of 70 eV, an ionization current of 300 μA and an accelerating voltage of 3 kV.

RESULTS

The profile of organic acids in 5 mg of non-ischemic rabbit renal tissue is shown in Fig. 1 (upper chromatogram). Some thirty compounds were detected in the gas chromatogram. Identification of these peaks was performed by comparing their mass spectra and retention times with those of authentic compounds or from references in the literature.

The change of organic acids in the renal tissue was determined by the time

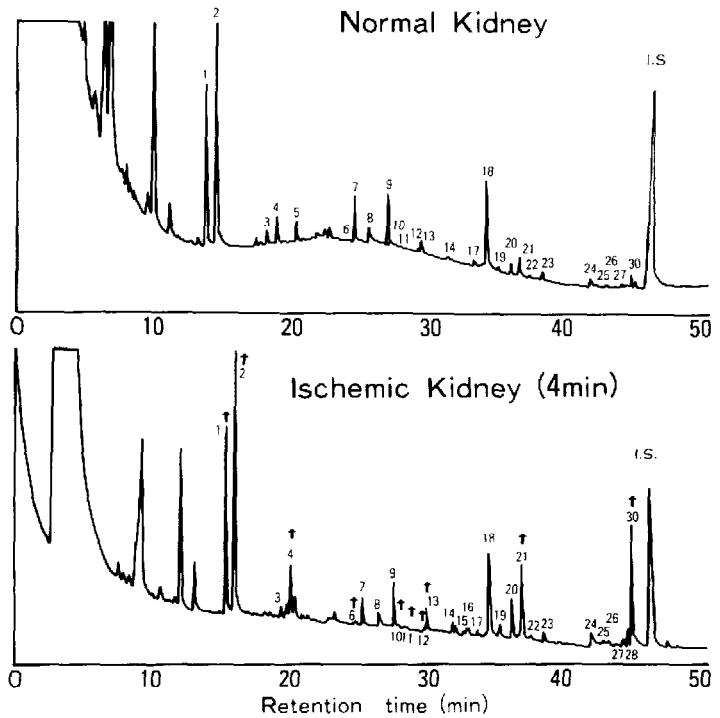

Fig. 1. Gas chromatograms of trimethylsilyl derivatives of organic acids from 5 mg of renal tissue biopsied before clamping the renal artery and vein (upper chromatogram) and 4 min after clamping (lower chromatogram). The biopsied samples were immediately frozen. The peaks identified were as follows: 1 = lactic acid, 2 = glycolic acid, 3 = 2-hydroxybutyric acid, 4 = 3-hydroxypropionic acid, 5 = 3-hydroxybutyric acid, 6 = 4-hydroxybutyric acid, 7 = diethyleneglycol, 9 = glycerol, 10 = 4-hydroxy-2-butenoic acid, 11 = succinic acid, 12 = 2-methylglyceric acid, 13 = glyceric acid; 14 = 3-deoxy-2-C-hydroxymethyltetronol-1,4-lactone, 17 = 3-deoxytetronic acid, 18 = 2-deoxytetronic acid, 21 = malic acid, 23 = 2,3-dideoxypentonic acid, 27 = 3-deoxy-2-C-hydroxymethyltetronic acid, 28 = 3-deoxypentonic acid, 29 = 3-deoxy-2-C-hydroxymethylpentono α-1,4-lactone, 30 = 3-deoxy-2-C-hydroxymethylpentono β-1,4-lactone. I.S. = internal standard. The peaks indicated by arrows in the lower chromatogram were increased in intensity compared with the upper control gas chromatogram.

elapsed after clamping the renal artery and vein to examine the ischemic metabolic change. The gas chromatogram of the organic acids in the ischemic renal tissue obtained 4 min after clamping is shown in Fig. 1 (lower chromatogram). Many peaks were increased over the control level, especially those of lactic acid, glycolic acid, 3-hydroxypropionic acid, glyceric acid, and malic acid. The levels of these organic acids, however, decreased in the ischemic renal tissue 15 min after clamping.

Fig. 2 shows the change of lactic acid, glycolic acid, 2-hydroxybutyric acid, 3-hydroxypropionic acid, 4-hydroxybutyric acid, and glycerol after clamping renal vessels. It can be seen that lactic acid, glycolic acid, and 2-hydroxybutyric acid increased in the renal tissue at 4 and 6 min after clamping, but then decreased from 15 min after clamping. 4-Hydroxybutyric acid increased in the renal tissue from 10 to 30 min after clamping. Glycerol increased in the renal tissue at 2 min after clamping but decreased from 6 min after clamping.

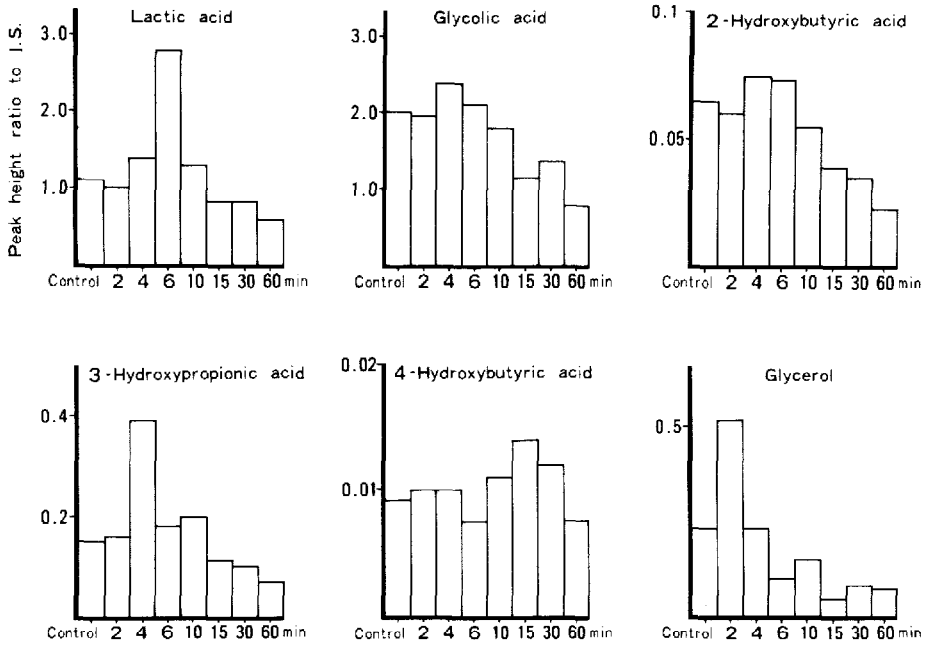

Fig. 2. The change in lactic acid, glycolic acid, 2-hydroxybutyric acid, 3-hydroxypropionic acid, 4-hydroxybutyric acid, and glycerol in renal tissue after clamping the renal artery and vein. Each bar represents the value averaged from five specimens. The abscissa represents 2 min, 4 min, 6 min, 10 min, 15 min, 30 min, and 60 min time elapsed, and the ordinate represents peak height ratio with respect to an internal standard.

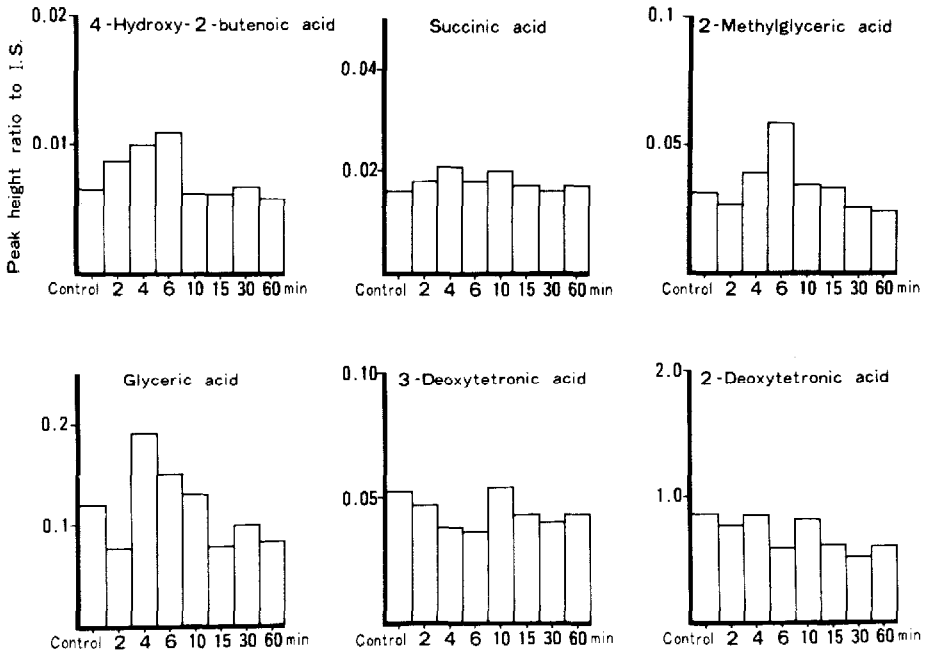

Fig. 3. The change in 4-hydroxy-2-butenoic acid, succinic acid, 2-methylglyceric acid, glyceric acid, 3-deoxytetronic acid, and 2-deoxytetronic acid in renal tissue after clamping the renal vessels.

Fig. 3 shows the change of 4-hydroxy-2-butenoic acid, succinic acid, 2-methylglyceric acid, glyceric acid, 3-deoxytetronic acid, and 2-deoxytetronic acid in the renal tissue after clamping renal vessels. 4-Hydroxy-2-butenoic acid increased in the renal tissue from 2 to 6 min after clamping, but then remained at around the control level. Succinic acid increased slightly in the renal tissue after clamping. 2-Methylglyceric acid and glyceric acid increased at 4 and 6 min after clamping and then decreased. 3-Deoxytetronic acid decreased in the renal tissue after clamping except at 10 min. 2-Deoxytetronic acid slightly decreased in the renal tissue after clamping.

Fig. 4 shows the change of malic acid, 2,3-dideoxypentonic acid, 3-deoxy-2-C-hydroxymethyltetronic acid, and 3-deoxypentonic acid in the renal tissue after clamping renal vessels. Malic acid increased at 4 and 6 min after clamping, but then decreased slightly. 2,3-Dideoxypentonic acid increased at 2 min after clamping, but then remained at around the control level. 3-Deoxy-2-C-hydroxymethyltetronic acid and 3-deoxypentonic acid decreased in the renal tissue after clamping.

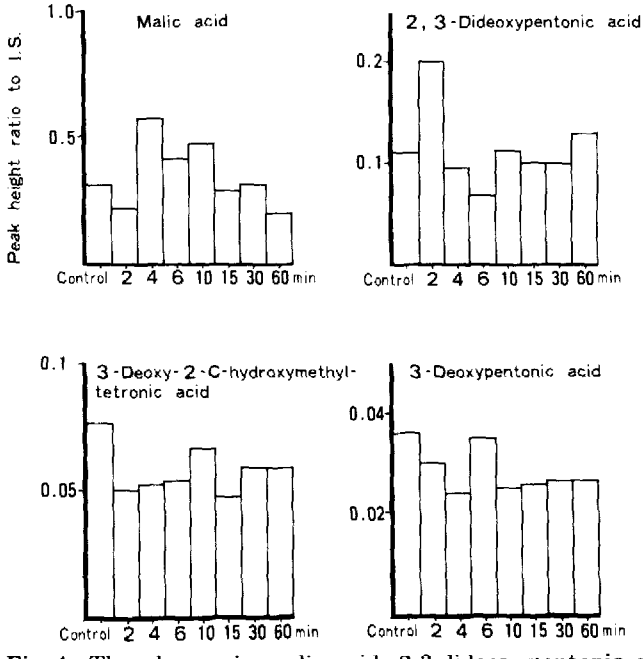

Fig. 4. The change in malic acid, 2,3-dideoxypentonic acid, 3-deoxy-2-C-hydroxymethyltetronic acid, and 3-deoxypentonic acid in renal tissue after clamping the renal vessels.

DISCUSSION

Profiling analysis of the organic acids in the renal tissue specimen was used effectively to identify the compounds and to examine the change in the compounds under ischemic conditions.

Lactic acid, glycolic acid, 3-hydroxypropionic acid, 2-methylglyceric acid, glyceric acid, and malic acid increased at 4 and 6 min after clamping renal vessels, and then decreased. Lactic acid is well known to increase in the hypoxic

condition, due to the increased NADH/NAD$^+$ ratio. Glycolic acid is formed from reduction of glyoxylic acid or glycol aldehyde by the action of glyoxylic reductase or glycol aldehyde dehydrogenase, respectively. The increase of glycolic acid in the renal tissue at 4 and 6 min after clamping would appear to reflect the increase of the NADH/NAD$^+$ ratio in the hypoxic state. Our observation that lactic acid and glycolic acid increased in an early period of ischemia is consistent with findings reporting [3] that both acids increase in the rat heart muscle in early ischemia. 2-Hydroxybutyric acid is also known to change in parallel to lactic acid. In lactic acidosis the urinary excretion of 2-hydroxybutyric acid is increased [5, 6]. 2-Hydroxybutyric acid is derived from 2-ketobutyric acid by the action of a subfraction of lactate dehydrogenase [7]. The increase of 2-hydroxybutyric acid may be related to the increased NADH/NAD$^+$ ratio. 3-Hydroxypropionic acid is derived from malonic acid semialdehyde by the action of 3-hydroxypropionic dehydrogenase. The increase of 3-hydroxypropionic acid in an early period of ischemia may also be due to the increased NADH/NAD$^+$ ratio in hypoxia. Glyceric acid is formed from hydroxypyruvic acid by the action of gyceric dehydrogenase. The increase of glyceric acid in early ischemia may again reflect an increased NADH/NAD$^+$ ratio. Although 2-methylglyceric acid changed in parallel to lactic acid, the metabolic pathway for the formation of 2-methylglyceric acid is not yet known.

Glycerol rapidly increased in the renal tissue after clamping and then decreased at 6 min. Succinic acid increased slightly in ischemic renal tissue. This finding is in contrast to the marked increase of succinic acid in hypoxic heart muscle [8].

3-Deoxyaldonic acids, such as 3-deoxytetronic acid, 3-deoxy-2-C-hydroxymethyltetronic acid, and 3-deoxypentonic acid, decreased in the renal tissue after clamping. This result is also in contrast to the report that these acids increased in heart muscle in the early period of ischemia, and then decreased [3]. The metabolism of these deoxyaldonic acids in the kidney may be different from that in the heart.

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