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Movement of Subcellular Calcium in the Liver of Bile Duct-ligated Rats

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The movement of subcellular calcium in the liver was investigated after a single intraperitoneal administration of calcium chloride to bile duct-ligated rats. The hepatic mitochondrial and microsomal calcium levels significantly increased 10 min after the administration of calcium (4.0 and 15.0 mg Ca/100 g body weight) to sham-operated rats, and began to decrease 30 and 60 min after that time. However, those reduction were clearly prevented by ligation of the bile duct. The administration of calcium (15.0 mg Ca/100 g) to bile duct-ligated rats induced a marked accumulation of calcium in the mitochondria as compared with the microsomes. Meanwhile, the cytosolic calcium was not significantly increased by the administration of calcium (4.0 and 15.0 mg Ca/100 g). The present results suggest that the cytosolic calcium taken up by the liver cells is largely regulated by accumulation into the mitochondria and transportation into the biliary duct of rat liver.

Keywords—calcium; hepatic bile; mitochondria; microsome; cell calcium metabolism

It is well known that the bone, kidney and intestine are the regulatory organs of calcium metabolism in mammals. Recently, it has been reported that the liver participates in the regulation of calcium metabolism.¹⁻³⁾ Calcitonin, a calcium-regulating hormone, increases the calcium content in the liver¹⁾ and stimulates calcium excretion into the bile of rats.^{2,3)} These investigations demonstrated that calcium excretion into the bile through the liver from the blood plays a physiological role in the control of calcium metabolism. However, the metabolism of intracellular calcium in the liver is not yet fully understood.

Therefore, the present study was undertaken to examine the subcellular movement of calcium taken up by the liver cells after calcium administration to bile duct-ligated rats. We found that the calcium taken up by the liver cells is markedly accumulated in the mitochondria.

Materials and Methods

Animals—Male Wistar rats, weighing approximately 120 g, were used. The animals were fed commercial laboratory chow containing 1.1% calcium and 1.1% phosphate (Oriental Test Diet Co., Ltd., Tokyo) and tap water *ad libitum*.

Drug—Calcium chloride dissolved in demineralized water (4.0 and 15.0 mg Ca/ml) was given by a single intraperitoneal injection (1.0 ml/100 g body weight) to rats. Demineralized water was injected as a control.

Surgical Procedure—The abdomen was opened by a midline incision under light ether anesthesia. The common bile duct was then ligated, and the incision was closed with wound clips. The animals were put into a cage for 1 h, and they were not fed or given water. The sham-operated rats did not receive bile duct ligation, although the abdomen was opened by a midline incision.

One hour after ligation of the bile duct, the rats were intraperitoneally administered calcium chloride (4.0 and 15.0 mg Ca/100 g), and killed after various times.

Analytical Methods—The rats were bled by cardiac puncture under light anesthesia with ether. The liver was perfused with a cold 0.25 M sucrose solution (10 ml) after bleeding and immediately removed. The liver was homogenized in 4 volumes of 0.25 M sucrose solution in a Potter-Elvehjem homogenizer with a Teflon pestle, and fractionated by differential centrifugation.⁴⁾ The homogenate was spun at $120 \times g$ in a refrigerated centrifuge for 10 min and then the supernatant was spun at $1100 \times g$. The $1100 \times g$ supernatant

was spun at $10800 \times g$ for 10 min, and the precipitate (ppt; mitochondrial fraction) was collected. Then the $10800 \times g$ supernatant was spun at $105000 \times g$ for 60 min. The precipitate (microsome fraction) and the supernatant (cytosol fraction) were separated. The amounts of calcium in the liver tissue and each subcellular fraction were determined by atomic absorption spectrophotometry after digestion with nitric acid. The concentration of protein was measured by the method of Lowry *et al.*⁵⁾

Statistical Methods—The significance of the differences between values was estimated by Student's *t* test. *p* values of less than 0.01 were considered to indicate statistically significant differences.

Results

The time courses of alterations of liver calcium after a single intraperitoneal administration of calcium chloride to bile duct-ligated rats are shown in Fig. 1. The liver calcium in sham-operated rats increased significantly 10 min after the administration of calcium (4.0 mg Ca/100 g), and then began to decrease rapidly. Meanwhile, the liver calcium in bile duct-ligated rats increased significantly during 10 min after the administration of calcium, and gradually after that time (Fig. 1A). Thus, ligation of the bile duct prevented the decline of liver calcium after its increase by calcium administration. This effect was markedly enhanced at a higher dose of calcium (15.0 mg Ca/100 g) (Fig. 1B).

The time courses of alterations of the calcium content in the subcellular fraction of liver after a single intraperitoneal administration of calcium chloride to bile duct-ligated rats are shown in Figs. 2 and 3. As early as 10 min after the administration of calcium (4.0 mg Ca/100 g) to sham-operated rats, the mitochondrial and microsomal calcium increased (Figs. 2A and 3B). The mitochondrial and microsomal calcium reached a maximum at 10 min, and decreased gradually after that time. However, with a higher dose (15 mg Ca/100 g), the mitochondrial calcium level was significantly increased at 10 and 30 min after calcium administration, while the microsomal calcium was not elevated significantly (Figs. 2B and 3B).

On the other hand, ligation of the bile duct clearly prevented the decline of the mitochondrial and microsomal calcium levels after the increase caused by the administration of calcium (4.0 mg Ca/100 g) to sham-operated rats (Figs. 2A and 3A). A remarkable elevation of liver calcium produced by calcium (15 mg Ca/100 g) administration to bile duct-ligated rats induced a marked elevation of the mitochondrial calcium as compared with the microsomal calcium (Figs. 2B and 3B). Thus, the mitochondria accumulated intracellular calcium more effectively than the microsomes of the liver cells.

The cytosolic calcium content in the liver cells of sham-operated rats was 0.52 ± 0.08 nmol/mg protein. This value was not significantly changed at any time after the administration of calcium (4.0 and 15.0 mg Ca/100 g) to sham-operated rats and bile duct-ligated rats.

Fig. 1. Changes of Liver Calcium after the Administration of Calcium Chloride to Bile Duct-ligated Rats

The rats received a single intraperitoneal administration of calcium chloride (4.0 and 15.0 mg Ca/100 g) 1 h after ligation of the bile duct or the sham operation. Each point represents the mean of 5 animals. Vertical lines represent the S.E.

a) $p < 0.01$ as compared with the zero time group.

b) $p < 0.01$ as compared with the sham operation group.

A; dose of 4.0 mg Ca/100 g, B; dose of 15.0 mg Ca/100 g.

—○—; sham-operated rats, —●—; bile duct-ligated rats.

Fig. 2. Changes of Hepatic Mitochondrial Calcium after the Administration of Calcium Chloride to Bile Duct-ligated Rats

The rats received a single intraperitoneal administration of calcium chloride (4.0 and 15.0 mg Ca/100 g) 1 h after ligation of the bile duct or the sham operation. Each point represents the mean of 5 animals. Vertical lines represent the S.E.

a) $p < 0.01$ as compared with the zero time group.

b) $p < 0.01$ as compared with the sham operation group.

A; dose of 4.0 mg Ca/100 g, B; dose of 15.0 mg Ca/100 g.

—○—; sham-operated rats, —●—; bile duct-ligated rats.

Fig. 3. Changes of Hepatic Microsomal Calcium after the Administration of Calcium Chloride to Bile Duct-ligated Rats

The rats received a single intraperitoneal administration of calcium chloride (4.0 and 15.0 mg Ca/100 g) 1 h after ligation of the bile duct or the sham operation. Each point represents the mean of 5 animals. Vertical lines represent the S.E.

a) $p < 0.01$ as compared with the zero time group.

b) $p < 0.01$ as compared with the sham operation group.

A; dose of 4.0 mg Ca/100 g, B; dose of 15.0 mg Ca/100 g.

—○—; sham-operated rats, —●—; bile duct-ligated rats.

Discussion

It was reported that a single intraperitoneal injection of calcium chloride (4.0 mg Ca/100 g body weight) to rats causes remarkable elevation of the calcium contents in the liver and bile, suggesting that the calcium taken up by the liver is excreted into the bile.³⁾ Thus, in the present study, we examined the movement of intracellular calcium in the liver of bile duct-ligated rats. The ligation of the bile duct significantly prevented the removal of the liver calcium increased by the administration of calcium (4.0 mg Ca/100 g). This effect was markedly enhanced at a higher dose (15.0 mg Ca/100 g). These results suggest that the bile excretion of calcium taken up by the liver cells plays a cell physiological role in maintaining the homeostasis of intracellular calcium.

The administration of calcium produced a significant increase in liver calcium and a corresponding elevation of the mitochondrial and microsomal calcium but not the cytosolic calcium. However, a higher dose of calcium caused calcium accumulation to increase more in the mitochondria than microsomes. Such accumulation in the mitochondria was markedly enhanced by ligation of the bile duct. Meanwhile, the cytosolic calcium was not significantly altered by ligation of the bile duct. From these results, it is possible that the concentration of calcium in the cytosol is largely maintained by the calcium accumulated in mitochondria rather than microsomes. The mitochondria may regulate the homeostasis of cytosolic concentration of calcium taken up by the liver cells.

The cytosolic calcium in the liver cells showed a constant level when calcium was administered intraperitoneally, suggesting that the movement of cytosolic calcium may be regulated by the following mechanism. The calcium that enters liver cells is firstly translocated into the cytosol, and then transported to the subcellular organelles and the biliary duct. This transportation process may be very prompt. Since calcium-binding protein exists in the cytosol of rat liver,⁶⁻⁸⁾ this protein may play a part in the intracellular transport of calcium

taken up by the liver cells. The cytosolic calcium movement in the liver cells remains to be elucidated.

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Comparative Immunological Studies of Mutarotases from Mammals

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The antibodies against purified rat and hog kidney mutarotases (type II) were prepared by injecting each of the purified mutarotases into rabbits and were found to quantitatively precipitate the enzyme activities of rat and hog kidney mutarotases, respectively. Immunological cross-reactivity among four forms of both rat and hog kidney mutarotases and among mutarotases from the kidney, liver, and small intestine of 5 species of animals, rat, hog, mouse, beef, and rabbit, was examined by using these antibodies. It was shown that the four forms of mutarotase in rat kidney as well as those in hog kidney were immunologically identical and that the kidney, liver, and small intestine of each species contain very similar enzymes, though there were clear differences between the enzymes from different species.

Keywords—mammalian mutarotases; multiple forms; anti-mutarotase antibody; immunological identity; double immunodiffusion test; immunoelectrophoresis; quantitative precipitin analysis

Introduction

Mutarotase (aldose 1-epimerase, EC 5.1.3.3) catalyzes the interconversion of the α - and β -anomers of D-glucose and other related sugars. It has been identified in a variety of species including mammals. Relatively high activity of the enzyme is contained in the kidney, liver and small intestine of mammals.¹⁾

Mulhern *et al.*²⁾ studied the physical characteristics of mutarotases from the kidneys of several animals and described that all of the mutarotases examined seemed to have similar molecular weights and similar shapes. Bailey *et al.*³⁾ reported a failure to produce the antibody against mutarotase from mammals. They attributed their failure to the close structural similarity of the enzymes in various mammals. Furthermore, despite extensive studies on