Movement of Subcellular Calcium in Bile Pool of Hepatocyte in Rats : Effect of Thyroparathyroidectomy

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Abstract

The movement of subcellular calcium in the bile pool of hepatocyte was investigated after a single intraperitoneal administration of calcium chloride in rats. The administration of calcium (4.0 mg/100 g BW) produced a remarkable elevation of serum calcium and a corresponding increase in liver calcium. The calcium taken by the liver cells at 10 min after calcium administration was markedly located into the nuclei, mitochondria and microsomes, and this distribution was not accompanied by a significant elevation in the cytosol level. At 20 min after calcium administration, the calcium increase above the subcellular structure was clearly reduced. On the other hand, serum calcium was markedly increased by calcium administration in both intact and thyroparathyroidectomized rats. However, the liver calcium increase induced in intact rats by calcium administration was much more than that in thyroparathyroidectomized rats. Also, the bile calcium level was markedly elevated by calcium administration in intact rats, but no elevation was observed in thyroparathyroidectomized rats. The present results suggest that the calcium taken by the liver cells is bound to the nuclei, mitochondria and microsomes, and then transported into the biliary duct.

It is well known that bone, kidneys and intestine are the regulatory organs of calcium metabolism in mammals. Recently, it has been reported that the liver participates in calcium metabolism. Calcitonin, a calcium-regulating hormone, increases the calcium concentration in the liver (Yamaguchi *et al.*, 1975) and stimulates the calcium excretion into the bile of rats (Yamaguchi and Yamamoto, 1978). From the results of these studies, it is suggested that the excretion of calcium into the bile through the liver from the blood plays a physiological role in the regulation of calcium metabolism. However, the metabolism of intracellular calcium in the liver is not yet fully understood. The

study was therefore undertaken to investigate the correlation between calcium metabolism in the liver cells and calcium excretion into the bile of rats intraperitoneally administered calcium chloride. We found that the calcium taken by the liver cells is excreted into the bile.

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 was dissolved in demineralized water to concentrations of 1.0, 2.0 and 4.0 mg Ca/ml.

This solution was given in a single intraperitoneal administration (1 ml/100 g BW) to rats. Demineralized water was injected as a control.

Surgical Procedures

The thyroparathyroid gland complex was removed with fine forceps under light anesthesia with ether. Calcium chloride (4.0 mg/100 g) was intraperitoneally administered 24 hr after thyroparathyroidectomy. The rats were killed 10 min after the administration of calcium.

Under intraperitoneal 25% urethane anesthesia (0.6 ml/100 g), the abdomen was opened by a midline incision. The bile duct was then cannulated with PE-10 tubing, which was secured in place, and the incision was closed with wound clips. The animals were put on a warm water bath ($38 \pm 1^\circ\text{C}$) to maintain the body temperature (Robert *et al.*, 1967), and the bile was collected twice at 10 min-intervals. The rats were not fed or given water. The administration of calcium chloride (4.0 mg Ca/100 g) was carried out intraperitoneally at the midpoint of the abdomen.

Analytical Methods

The animals were bled by cardiac puncture under light anesthesia with ether. Blood samples obtained by cardiac puncture were centrifuged immediately after collection. The serum was separated and analysed immediately. Calcium in the serum was measured by atomic absorption spectrophotometry after precipitation with 10% trichloroacetic acid (Wilis, 1960).

Liver was perfused with a cold 0.25 M sucrose solution after bleeding and removed immediately. The liver tissue was suspended 1:4 in a 0.25 M sucrose solution and homogenized in a Potter-Elvehjem homogenizer with a Teflon pestle, and the subcellular fractions were isolated by differential centrifugation (Hogeboom, 1955). The amount of calcium in each fraction was determined by atomic absorption spectrophotometry after digestion with nitric acid. Protein was determined by the method of Lowry *et al.* (1951). The calcium content was expressed as nmol per mg protein.

The bile volume was measured by means of a pipet graduated in 0.01 ml. The amount of bile calcium was determined by atomic absorption spectrophotometry after precipitation with 10% trichloroacetic acid. The bile calcium content was expressed as the excreted calcium (μg) per 100 g BW of the rats.

Statistical Methods

The significance of the difference between the values was estimated by Student's *t*-test. *P* values less than 0.05 were considered to indicate a statistically significant difference.

Fig. 1. Effect of a single intraperitoneal administration of calcium chloride on the serum and liver calcium concentrations in rats. The rats were killed 10 min after the administration of calcium. Each point represents the mean for 5 animals. Vertical lines represent the SE. *, $p < 0.01$ as compared with control group. $\circ$ --- $\circ$; serum calcium, $\bullet$ — $\bullet$; liver calcium.

Results

The effect of increasing amounts of calcium chloride on the serum and liver calcium is shown in Fig. 1. The animals were killed 10 min after the administration of calcium chloride. The serum calcium was significantly increased by the administration of 2.0 and 4.0 mg Ca/100 g BW but not significantly by 1.0 mg Ca/100 g. The liver calcium was significantly elevated even at the lowest dose (1.0 mg Ca/100 g). With the higher doses, the liver calcium increased markedly.

The effect of increasing amounts of calcium chloride on the calcium content in the subcellular fraction of liver is shown in Table 1. The calcium content in the plasma membrane, nuclei, mitochondria and microsome fractions was significantly increased by

Table 1. Effect of the administration of calcium on calcium content in the subcellular fraction of rat liver

Subcellular fraction	Calcium content (nmol/mg protein)			
	Control	Dose of calcium (mg/100 g)		
		1.0	2.0	4.0
Plasma membrane	2.80±0.23	4.65±0.30 ^a	4.86±0.33 ^a	5.74±0.44 ^a
Nuclei	1.80±0.06	6.37±0.21 ^a	5.74±0.26 ^a	6.10±0.39 ^a
Mitochondria	2.38±0.08	3.91±0.28 ^a	3.68±0.27 ^a	5.39±0.45 ^a
Microsomes	2.47±0.17	3.87±0.23 ^a	4.43±0.49 ^a	5.45±0.56 ^a
Cytosol	0.57±0.08	0.48±0.11	0.52±0.10	0.82±0.06

Values are mean±SE for 6 animals.

The rats were killed 10 min after a single intraperitoneal administration of calcium chloride.

a; $p < 0.01$ as compared with control.

the administration of calcium (1.0, 2.0 and 4.0 mg Ca/100 g), while that in the cytosol fraction was not significantly elevated by any of the doses.

The time course of alteration of the serum and liver calcium after the administration of calcium chloride (4.0 mg Ca/100 g) is shown in Fig. 2. The results show that, as early as 10 min after calcium administration, there was the increase in serum and liver calcium. The serum and liver calcium reached a maximum at 10 min, and decreased rapidly 20 min after calcium administration. The liver calcium significantly increased even at 90 min after calcium administration.

The time course of alteration of the calcium content in the subcellular fraction of liver after the administration of calcium chloride (4.0 mg Ca/100 g) is shown in Table 2. The maximum increase in the calcium content in the plasma membrane, nuclei, mitochondria and microsome fraction was observed 10 min after calcium administration. The plasma membrane calcium returned to normal levels 20 min after calcium administration. The nuclei and mitochondria calcium began to decrease at 20 min after calcium administration, but significantly increased even at 90 min. The microsomes calcium increase was gradually reduced, and returned to the normal level 60 min after calcium administration. The cytosolic calcium was not significantly

Fig. 2. Time course of the alteration of the serum and liver calcium concentrations after a single intraperitoneal administration of calcium chloride in rats. The rats were killed at varying times after the administration of calcium (4.0 mg Ca/100 g). Each point represents the mean for 5 animals. Vertical lines represent the SE. *: $p < 0.01$ as compared with the values obtained at zero time. ○—○; serum calcium, ●—●; liver calcium.

Table 2. Time course of the alteration of calcium content in the subcellular fraction of rat liver after the administration of calcium

Subcellular fraction	Calcium content (nmol/mg protein)					
	0 min	10 min	20 min	40 min	60 min	90 min
Plasma membrane	2.78±0.25	5.86±0.56 ^a	3.19±0.05	3.18±0.31	2.72±0.29	2.89±0.30
Nuclei	1.91±0.08	6.12±0.36 ^a	4.19±0.40 ^a	3.73±0.41 ^a	3.75±0.42 ^a	3.72±0.36 ^a
Mitochondria	2.32±0.15	5.46±0.48 ^a	3.81±0.20 ^a	3.85±0.43 ^a	3.65±0.62 ^a	3.54±0.21 ^a
Microsomes	2.47±0.20	5.58±0.48 ^a	3.94±0.27 ^a	3.46±0.40 ^a	3.20±0.60	2.95±0.28
Cytosol	0.52±0.08	0.79±0.07	0.61±0.19	0.49±0.04	0.53±0.08	0.68±0.02

Values are mean±SE for 6 animals.

The rats received a single intraperitoneal administration of calcium chloride (4.0 mg Ca/100 g).

a; $p < 0.01$ as compared with control.

Table 3. Effect of thyroparathyroidectomy on the serum and liver calcium concentration after the administration of calcium

Treatment	Serum calcium (mg/100 ml)		Liver calcium (μ g/g wet tissue)	
	4 min	10 min	4 min	10 min
Intact rats				
Control	9.60±0.10	9.58±0.06	15.52±1.10	15.00±0.94
Calcium	14.66±0.30 ^a	16.06±0.62 ^a	29.70±1.82 ^a	38.66±1.25 ^a
Thyroparathyroidectomized rats				
Control	7.12±0.23	7.08±0.20	21.01±1.86	20.84±1.43 ^c
Calcium	11.64±0.83 ^a	13.25±0.67 ^a	23.14±1.55	26.05±1.03 ^{a, b}

Values are mean±SE for 6 animals.

The rats were thyroparathyroidectomized 24 hr before the experiment.

The rats were killed 4 and 10 min after a single intraperitoneal administration of calcium chloride (4.0 mg Ca/100 g).

a; $p < 0.01$ as compared with control.

b; $p < 0.01$ as compared with intact rats administered calcium.

c; $p < 0.01$ as compared with intact rats.

increased by the administration of calcium.

The effect of thyroparathyroidectomy (TPTX) on the changes in serum and liver calcium after calcium (4.0 mg Ca/100 g) administration was examined (Table 3). Calcium administration in intact rats caused a marked increase in both serum and liver calcium. TPTX caused a remarkable fall in serum calcium of intact rats, while liver calcium was increased. The administration of calcium in thyroparathyroidectomized rats produced a marked augmentation of serum calcium and a slight but significant elevation of liver calcium. However, the degree of increase in liver calcium caused by calcium administration in thyroparathyroi-

dectomized rats was much less than that in intact rats.

The change in bile calcium after the administration of calcium chloride (4.0 mg Ca/100 g) is shown in Fig. 3. The amount of bile calcium was not significantly increased 10 min after calcium administration in intact rats, and then it was markedly elevated between 10 and 20 min after calcium administration. In the thyroparathyroidectomized rats, on the other hand, the calcium administration had no effect on the bile calcium excretion.

Fig. 3. Change in calcium excretion into the bile after a single intraperitoneal administration of calcium chloride in intact and thyroparathyroidectomized rats. The rats were thyroparathyroidectomized 24 hr before the experiment. The bile was collected twice at 10 min-intervals after the administration of calcium (4.0 mg Ca/100 g). Each bar represents the mean for 5 animals. Vertical lines represent the SE. *: $p < 0.01$ as compared with control group. □; control, ■; calcium administration.

Discussion

We reported earlier that calcitonin (CT), a calcium-regulating hormone, causes an increase in calcium in the liver (Yamaguchi *et al.*, 1975) and a stimulation of calcium excretion into the bile of rats (Yamaguchi and Yamamoto 1978). More recently we suggested that endogenous CT regulates calcium metabolism in the hepatic bile system of rats (Yamaguchi, 1980). However, the movement of subcellular calcium in the bile pool of hepatocyte had not been fully understood.

In the present study, the alteration in the amount of calcium in the subcellular fraction of the liver was investigated after a single intraperitoneal administration of calcium chloride in rats. The administration of calcium produced a remarkable increase in serum calcium and a corresponding elevation of liver calcium. The calcium increased in the liver cells at 10 min after calcium administration was markedly located in the subcellular construction, i.e. nuclei, mitochondria and microsomes, although the calcium in the cytosol was not increased significantly. These results indicate that the calcium taken by the liver cells was rapidly sequestered by the subcellular construction. However, calcium, which increased in the subcellular construc-

tion 10 min after calcium administration, was markedly decreased 20 min after that time. This fact shows that an increase in calcium in the subcellular construction after calcium administration was rapid, but temporary. It appears that the nuclei, mitochondria and microsomes promptly sequester calcium which entered the liver cells to maintain homeostasis of calcium in the cytosol, and after that the calcium is released.

On the other hand, thyroparathyroidectomy (TPTX) caused a fall in serum calcium and a slight increase in liver calcium. The decrease in serum calcium after TPTX may result from the depletion of parathyroid hormone (PTH) which induces the elevation of the serum calcium level. TPTX may also increase liver calcium, since Ca-ATPase activity in the plasma membrane of rat liver which is related to calcium efflux from intracellular to extracellular is decreased by TPTX (Yamaguchi, 1980). Presumably, an increase in liver calcium caused by TPTX is not dependent on the action of CT or PTH from thyroid or parathyroid glands.

The serum calcium in intact and thyroparathyroidectomized rats was markedly elevated by calcium administration. However, the liver calcium increase following calcium administration in intact rats was much more than that in thyroparathyroidectomized rats. Thus an inhibitory effect of

TPTX on the increase in liver calcium after calcium administration is not dependent on a change in serum calcium. These results suggest that endogenous CT mainly participates in an increase in liver calcium after calcium administration, since a rapid increase in serum calcium mainly causes a secretion of CT from thyroid glands to regulate calcium homeostasis in the serum of rats (Broulik and Pocorsky, 1973). In fact, CT increases liver calcium in intact and thyroparathyroidectomized rats (Yamaguchi *et al.*, 1975; Yamaguchi, 1980). It is assumed that the effect of endogenous CT on liver calcium is exhibited rapidly after calcium administration.

The bile calcium was not significantly increased 10 min after calcium administration but was markedly elevated between 10 min after calcium administration, but was markedly elevated between 10 and 20 min. This increase was not produced in thyroparathyroidectomized rats, suggesting that endogenous CT induces an excretion of calcium into the bile, since it was previously reported that an increase in bile calcium excretion is prevented by TPTX and is markedly restored by CT (Yamaguchi and Yamamoto, 1978; Yamaguchi, 1980).

Moreover, the liver calcium increased at 10 min after calcium administration was markedly lowered at 20 min. These results indicate that the excretion of calcium into the bile was followed the an uptake by the liver cells.

From the present investigation, it is assumed that the calcium taken by the liver cells due to an increase in serum calcium is promptly sequestered by the nuclei, mitochondria and microsomes, and is then transported into the biliary duct.

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