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Dietary and Thyroparathyroidal Regulation of Calcium Excretion into the Bile of Rats

MASAYOSHI YAMAGUCHI* and TAKEO YAMAMOTO

Department of Environmental Biochemistry, Shizuoka College of Pharmacy, 2-2-1, Oshika, Shizuoka, 422, Japan

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The effects of fasting and thyroparathyroidectomy on bile calcium excretion in rats were investigated. The bile calcium excretion in rats fasted for 24 hr decreased slightly in comparison with that of fed rats. A single intraperitoneal administration of calcium chloride (4.0 mg Ca/100 g) to fed rats produced a remarkable elevation of bile calcium excretion, while a significant increase was not observed in fasted rats. On the other hand, thyroparathyroidectomy caused a significant reduction of bile calcium excretion in both fed and fasted rats. This reduction was much more pronounced in fasted rats than in fed rats. In thyroparathyroidectomized rats, a single intraperitoneal administration of calcium chloride (4.0 mg Ca/100 g) in both fed and fasted rats did not produce a significant elevation of bile calcium excretion. These results suggest that the bile calcium excretion is regulated by dietary calcium, as well as by the thyroid and parathyroid glands.

Keywords——calcium; calcium regulation; bile calcium; thyroparathyroid glands; fasted rats

It is well known that the bone, kidney, and intestine are the regulatory organs of calcium metabolism in mammals. We have recently reported that the bile pool of hepatocytes participates in the regulation of calcium metabolism.[1,2] Calcium in the body is excreted more extensively into the feces through the bile than into the urine in rats.[1] Ligation of the bile duct produces a marked elevation of calcium concentration in the serum of rats orally administered with calcium chloride.[2] Thus, the excretion of calcium into the bile may play a physiologi cal role in the regulation of serum calcium level after the ingestion of high calcium-containing diets. The present study was therefore undertaken to investigate the regulatory mechanisms of calcium excretion into the bile of rats. We found that the bile calcium excretion is regulated by dietary calcium, as well as by the thyroid and parathyroid glands.

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 freely.

Drug——Calcium chloride was dissolved in demineralized water (4.0 mg Ca/ml). This solution (1 ml/100 g body weight) was administered intraperitoneally. The vehicle was injected as a control.

Surgical Procedures——The thyroparathyroid gland complex was removed with fine forceps under light anesthesia with ether. Under intraperitoneal 25% urethane anesthesia (0.6 ml/100 g) at 24 hr after thyroparathyroidectomy, the abdomen was opened by 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 ($38\pm1°$) to maintain the body temperature,[3] and the bile was collected. 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. The bile was collected immediately after the administration of calcium.

Analytical Methods——The bile volume was measured by means a pipet graduated in units of 0.01 ml. The amount of calcium was determined by atomic absorption spectrophotometry (Perkin-Elmer, model 303) after precipitation with 10% trichloroacetic acid.[4] The bile calcium concentration was expressed in two ways: (i) the amount of bile calcium, defined as the excreted calcium (μg) per 100 g body weight of the rat; and (ii) the concentration of bile calcium, defined as calcium(μg) per milliliter of bile.

Statistical Methods——The data were subjected to an analysis of variance, and standard errors (SE) were calculated from the residual error term. The significance of the differences between values was estimated by Student's t test. p values less than 0.05 were considered to indicate statistically significant differences.

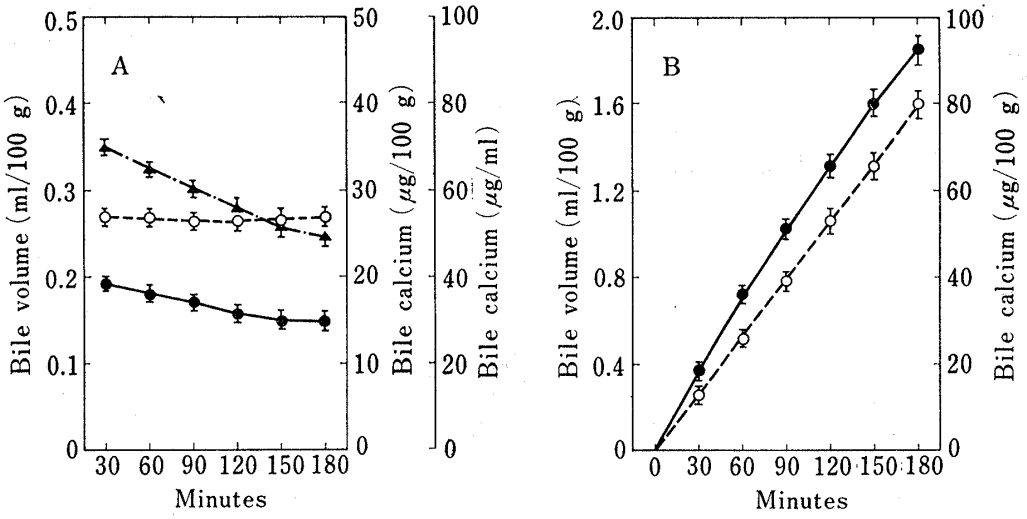

Fig. 1. Time Course of Calcium Content excreted into the Bile of Rats

Rats were anesthetized with a single intraperitoneal administration of 25% urethane solution. The bile was collected at 30-min intervals. Each point represents the mean of 5 animals. Vertical lines represent the SE. --○—; bile volume (ml/100 g), —●— ; bile calcium (μg/100 g), —·—▲—·—; bile calcium (μg/ml).

Results

The time course of calcium excretion into the bile of rats is shown in Figs. 1A and 1B. The bile was collected 6 times at 30 min intervals. The calcium content in the bile of rats gradually decreased during the collection period, while the bile volume was constant (Fig. 1A). When the bile volume and the bile calcium content excreted at each 30 min interval were accumulated, a linear increase of the bile volume was apparent, but the bile calcium was not

Table I. Effects of Fasting and Thyroparathyoidectomy on Calcium Excretion into the Bile of Rats after the Administration of Calcium Chloride

Treatment[a]		Number of rats	Bile volume[b] (ml/100 g)	Bile calcium content[b] (μg/100 g)	Bile calcium content[b] (μg/ml)
Intact rats	Fed rats				
	Control	5	1.60±0.14	90.8±4.1	60.6±5.1
	Calcium	6	1.58±0.12	143.8±8.8[c]	90.5±4.7[c]
	Fasted rats				
	Control	5	1.26±0.08[c]	72.3±3.9[c]	62.3±2.8
	Calcium	6	1.25±0.15	80.7±7.3	72.0±4.5
Thyroparathyroidectomized rats	Fed rats				
	Control	5	1.42±0.11	60.4±6.9[c]	45.2±2.9[c]
	Calcium	6	1.31±0.18	68.8±6.9	50.9±4.8
	Fasted rats				
	Control	5	0.96±0.07	29.9±1.6[c]	31.2±2.2[c]
	Calcium	6	0.98±0.08	32.1±2.0	35.1±3.1

a) Rats were thyroparathyroidectomized 24 hr before the experiments, and they were fasted for 24 hr. Rats received a single intraperitoneal administration of calcium chloride (4.0 mg Ca/100 g) immediately after the cannulation. The hile was collected for 3 hr.
b) Each value represents the mean ±S.E.
c) $p<0.01$ as compared with the control groups of intact fed rats.

related to it by a constant factor (Fig. 1B). Thus, the bile calcium content may be independent of the bile flow.

The effects of fasting and thyroparathyroidectomy (TPTX) on calcium excretion into the bile of rats shown in Table I. In intact rats, the bile volume and its calcium content (μg/100 g) were significantly decreased by fasting in comparison with those of control rats. When a solution of calcium chloride (4.0 mg Ca/100 g) was administered intraperitoneally to fed rats, the bile calcium content was markedly elevated in comparison with that of control rats. However, the administration of calcium to fasted rats did not cause a significant increase in the bile calcium content. Thus, it seems that a mechanism is operating in the fasted rats that prevents the excretion of calcium into the bile even after the administration of calcium.

In thyroparathyroidectomized rats, the bile volumes in fed and fasted rats slightly decreased in comparison with those of intact rats. The decrease of bile calcium content (μg/100 g) caused by TPTX was about 33% in fed rats as compared with intact fed control rats, while it was about 59% in fasted rats. A single intraperitoneal administration of calcium chloride (4.0 mg Ca/100 g) in both fed and fasted rats from which the thyroparathyroid glands had been removed did not produce any significant alteration of bile volume or bile calcium content in comparison with those of control rats. Thus, the augmentation of calcium excretion into the bile of fed rats was completely prevented by TPTX. However, this prevention was not reversed by the administration of calcium.

Discussion

The present study showed that fasting produced a significant decrease of calcium excretion into the bile of rats, indicating that dietary calicium may be excreted into the bile. Furthermore, a single intraperitoneal administration of calcium chloride in fed rats caused a remarkable elevation of bile calcium excretion, while such administration to fasted rats did not produce a significant augmentation. Thus, when calcium availability in rats is reduced by decrease of the intestinal calcium absorption from the diet, the administration of calcium chloride does not induce a marked elevation of calcium excretion into the bile in fasting rats. The bile calcium excretion is presumably regulated by fasting, probably by the levels of dietary calcium.

Thyroparathyroidectomy (TPTX) produced a marked decrease of calcium excretion into the bile of both fed and fasted rats. This decrease was much greater in fasted rats than in fed rats. Further, in thyroparathyroidectomized rats, the administration of calcium chloride did not cause a significant increase in calcium excretion into the bile even in fed rats. These results suggest that the bile calcium excretion is regulated by the thyroid and parathyroid glands, and this regulation may be predominant mainly in the fed state rather than the fasted state.

Calcitonin and parathyroid hormone, which are calcium-regulating hormones, are secreted from the thyroid and parathyroid glands, respectively. Calcitonin has a hypocalcemic effect,[5] while parathyroid hormone has a hypercalcemic effect.[6] Parathyroid hormone is secreted from the parathyroid gland in order to elevate lowered calcium levels in the serum of rats.[7] In contrast, calcitonin is secreted from the thyroid glands in response to an increase in serum calcium levels of rats.[8] Recently it has been reported that the calcitonin level in the plasma of rats rises rapidly after the start of the feeding period.[9] In the present work, the bile calcium excretion in fed rats was markedly increased by calcium administration, and this increase was completely prevented by TPTX. It is possible that calcitonin participates in calcium excretion into the bile.

The results of the previous[1,2] and present studies strongly suggest that the excretion of calcium into the bile plays a physiological role in the regulation of serum calcium level in response to the amount of calcium in the diet.

References and Notes

1) M. Yamaguchi, T. Yamamoto, and A. Hasegawa, *Chem. Pharm. Bull.*, **27**, 3137 (1979).
2) M. Yamaguchi and T. Yamamoto, *Chem. Pharm. Bull.*, **26**, 2568 (1978).
3) R.J. Robert, C.D. Klaassen, and G.L. Plaa, *Proc. Soc. Expt. Med. Biol.*, **125**, 313 (1967).
4) J.B. Willis, *Nature* (London), **16**, 249 (1960).
5) P.F. Hirsch, E.F. Voelkel, and P.L. Munson, *Science*, **146**, 412 (1963).
6) A.D. Care, L.M. Sherwood, J.T. Potts, Jr., and G.D. Aurbach, *Nature* (London), **209**, 55 (1966).
7) H. Rasmussen, *Amer. J. Med.*, **50**, 567 (1971).
8) P.D. Broulik and V. Pocorsky, *Endocrinol. Exp.*, **7**, 183 (1973).
9) R.V. Talmage, S.H. Doppelt, and C.W. Cooper, *Proc. Soc. Exp. Med. Biol.*, **149**, 855 (1975).

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The Use of Liposomes as Enzyme Carriers. I. Dependence of Enzyme Stability on the Method of Preparation

Yuji Kurosaki, Toshikiro Kimura, Shozo Muranishi, and Hitoshi Sezaki*

Faculty of Pharmaceutical Sciences, Kyoto University, Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto, 606, Japan

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The stability of liposomal enzymes and the encapsulation efficiency of reverse-phase evaporation vesicles (REV) were investigated in comparison with those of the original small multilamellar liposomes (SML), by using elastase as a model enzyme. The encapsulation efficiency of REV was greatly superior to that of SML. In addition, REV retarded the inactivation of elastase in the neutral pH region, as did SML. Therefore the REV method should be useful for the preparation of carriers to encapsulate not only elastase but also other macromolecules. However, at lower pH, pH 2.63, where elastase was rapidly inactivated, REV accelerated the inactivation of the enzyme, although SML retarded it, as at neutral pH. Thus, since liposomes prepared by different methods have significantly different physicochemical properties, further and more precise studies on the methods of preparation are required.

Keywords——enzyme; enzyme stability; elastase; liposomes; reverse-phase evaporation vesicles; encapsulation efficiency

The use of liposomes as a drug delivery system has been extensively investigated.[1] In the case of enzyme replacement therapy, deficient enzymes are often administered in the liposomal form so that the delivery of foreign enzymes to cells is facilitated while retaining their activities and avoiding immunological reactions.[2] One of the weak polints of liposomal preparations is low encapsulation efficiency due to the small internal aqueous space. Recently, Szoka and Papahadjopoulos reported a procedure for the preparation of reverse-phase evaporation vesicles (REV) with large internal aqueous space and high encapsulation efficiency.[3]

This report describes the stability of the liposomal enzymes and the encapsulation efficiency of REV in comparison with those of the original liposomal preparation of Bangham *et al.*[4] by using elastase as a model enzyme.

Experimental

Materials——Phosphatidylcholine was prepared from egg yolks according to the method of Rhodes and Lea.[5] Elastase and succinyl-tri-L-alanine-*p*-nitroanilide (STANA) were supplied by Eisai Co., Tokyo,