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Stimulatory Effect of Calcitonin on Calcium Excretion into Bile of Thyroparathyroidectomized Rats

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The effect of calcitonin (CT) on the bile calcium excretion in thyroparathyroidectomized rats has been investigated. A single intraperitoneal injection of calcium (4.0 mg/100 g) markedly increased the bile calcium excretion of sham-operated rats, suggesting that an increase in serum calcium elevates the excretion of calcium into the bile. In thyroparathyroidectomized rats, however, the injection of calcium did not significantly elevate the excretion of calcium into the bile. The bile calcium excretion of thyroparathyroidectomized rats injected with calcium was predominantly potentiated by the administration of CT. CT alone slightly increased the bile calcium excretion in thyroparathyroidectomized rats. In thyroparathyroidectomized rats injected with calcium, there was an increase in bile calcium excretion as early as 30 min after CT administration and the hormone markedly increased the bile calcium excretion even at the lowest dose (40 MRC mu/100 g). These results suggest that CT stimulates the excretion of calcium into the bile through the liver when calcium increases in the serum.

Keywords——calcitonin; bile calcium; calcium metabolism; stimulatory effect on bile calcium; thyroparathyroidectomized rats;

Introduction

Calcitonin (CT), a calcium-regulating hormone, exhibits a hypocalcemic effect by inhibiting calcium release from bone.[2,3] Recently it has been demonstrated that CT inhibits the calcium absorption from intestine.[4] Physiological significance of CT action has been evaluated with relation to an increase in serum calcium after the ingestion of high calcium-containing diet.

On the other hand, the bile is formed in the liver and secreted into the intestine. Previously it has been reported that the administration of a large dose of CT does not induce any alteration of the bile calcium level.[5] We showed, however, that CT produces calcium accumulation in the liver by inhibiting the efflux of calcium.[6,7] The behavior of calcium in enterohepatic system seems to be interesting for the effect of CT on calcium metabolism. The present study was therefore undertaken to investigate the effects of CT on calcium excretion into the bile of hydroparathyroidectomized rats. We found that CT increases the excretion of calcium into the bile.

Materials and Methods

Animals——Male Wistar rats, weighing approximately 120 g, were used throughout the study. The animals were fed commercial laboratory chow containing 7.4% carbohydrate, 1.1% calcium and 1.1% phosphate (Oriental Test Diet Co., Ltd., Tokyo) and tap water *ad libitum* until the day of testing.

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Drug and Hormone——Calcium chloride was dissolved in sterile, demineralized water. Calcitonin (lyophilized porcine calcitonin, 68 MRC U/mg, Armour Pharmaceutical Company, Kankakee, Ill.) dissolved in sterile, demineralized water. Control injections consisted of sterile, demineralized water.

Surgical Procedures——The thyroparathyroid gland complex was removed with fine forceps under light ether anesthesia and the incision closed with wound clips. The sham-operated animals underwent reanastomosis without removal of the glands. The animals were utilized 24 hr after thyroparathyroidectomy.

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 and it ligated, and the incision closed with wound clips. The animals put on the thermostatic water bath (38°±1°) in order to collect the bile[8] and were not fed or watered through the experiments. The administration of hormone was made subcutaneously at the midpoint of the abdomen. An injection of calcium chloride solution was done intraperitoneally at a separate site.

Analytical Methods——The bile was collected for thirty-minute periods for at least 180 min in each experiment. The bile bolume was measured by means of pipet graduated in 0.01 ml. The amount of bile calcium was determined by atomic absorption spectrophotometry (Perkin-Elmer, Model 303) with a reversed air-acetylene flame after precipitation with 10% trichloroacetic acid.[9] The bile calcium concentration was expressed in two ways: (i) amount of bile calcium, defined as the excreted calcium (μg) per 100 g body weights of rat; and (ii) content of bile calcium, defined as calcium (μg) per milliliter of bile.

Statistical Methods——The data were subjected to an analysis of the variance and standard errors (SE) were calculated from the residual error term. 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 statistically significant differences.

Results

The change of bile calcium after the cannulation with tubing is shown in Fig. 1. The amount of bile calcium in control rats gradually decreased at each time period. When the solution of calcium (2 or 4 mg/100 g) was administered intraperitoneally 60 min after the start of bile collection, the amount of bile calcium rapidly elevated 30 min after calcium injection, and then it decreased gradually. The bile in rats injected with 4 mg/100 g of calcium resulted in a significant ($p<0.01$) increase in calcium excretion greater than that observed with low calcium administration.

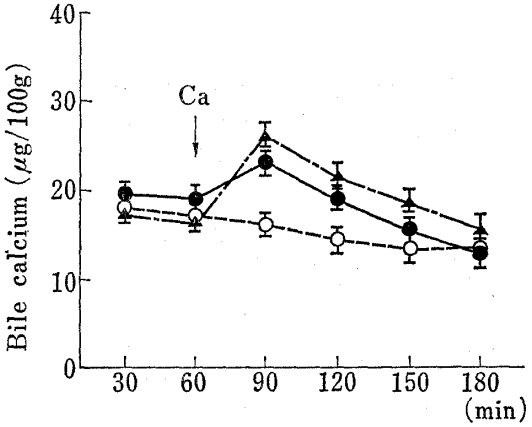

Fig. 1. Effect of Calcium Injection on the Bile Calcium Concentration in Rats

Rats were anesthetized with 25% urethane solution. Calcium (2.0 or 4.0 mg/100 g) was administered by a single intraperitoneal injection. The bile was collected for thirty minutes period. Each point represents the mean of 5 animals. Vertical lines represent the SE. ○----○; control, ●——●; calcium (2.0 mg/100 g), ▲——▲; calcium (4.0 mg/100 g).

The effect of calcitonin (CT) on the bile calcium excretion is shown in Table I. The animals were thyroparathyroidectomized 24 hr before the experiment. The bile collection commenced 90 min subsequent to hormone administration. The bile calcium concentration in the sham-operated rats was markedly potentiated by a single intraperitoneal injection of calcium (4.0 mg/100 g), and the bile volume was increased slightly. In the thyroparathyroidectomized rats, on the other hand, there was no effect of the calcium injection on the bile calcium concentration. Thus thyroparathyroidectomy inhibits only the calcium stimulable increase in bile calcium and not the entire excretion of calcium into the bile. Furthermore we investigated the influence of hormone. CT (80 MRC mU/100 g) was subcutaneously administered to thyroparathyroidectomized rats receiving calcium (4.0 mg/100 g) injected

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intraperitoneally. The administration of hormone did not show a significant alteration of bile volume. The administration of CT alone increased slightly the bile calcium concentration in thyroparathyroidectomized rats. When CT and calcium (4.0 mg/100 g) were administered simultaneously to thyroparathyroidectomized rats, the hormone administration produced a significantly greater bile calcium concentration of the calcium-injected rats than that of rats not receiving calcium. Thus CT stimulated the bile calcium excretion.

TABLE I. Effect of Calcitonin (CT) on the Bile Calcium Concentration in Rats after Thyroparathyroidectomy (TPTX)

Treatment	Number of rats	Bile volume (ml/100 g)	Bile calcium concentration (μg/100 g)	(μg/ml)
Sham(A)	5	0.61±0.04[a]	43.4±1.7	66.6± 6.9
Sham+Ca(B)	6	0.66±0.02	72.1±6.6[b]	109.6± 9.9[b]
(B)−(A)		0.05	28.7	43.0
TPTX(A)	5	0.64±0.02	39.2±3.3	61.4± 4.9
TPTX+Ca(B)	6	0.59±0.05	42.8±4.7	66.6± 5.8
(B)−(A)		−0.05	3.6	5.2
TPTX+CT(A)	5	0.67±0.03	55.3±7.9	82.7± 7.8
TPTX+Ca+CT(B)[c]	6	0.62±0.05	83.3±5.2[b]	134.8±10.8[b]
(B)−(A)		−0.05	28.0	52.1

a) Values are mean±SEM.
b) Significance from the (A), $p<0.01$ (Student's *t*-test).
c) Calcitonin (80 MRC mU/100 g) was administered subcutaneously 24 hr after thyroparathyroidectomy, and immediately calcium (4.0 mg/100 g) was injected intraperitoneally. The bile was collected for 90 min after the hormone administration.

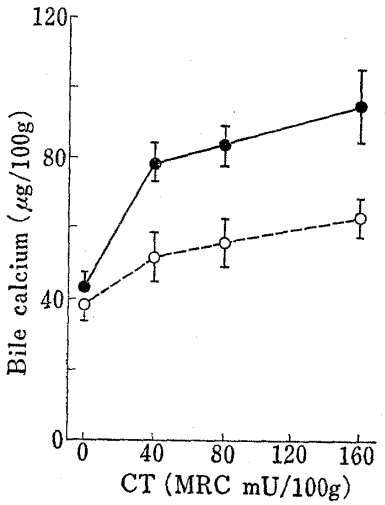

Fig. 2. Effect of Calcitonin on the Bile Calcium Concentration of Thyroparathyroidectomized Rats

Twenty-four hours after thyroparathyroidectomy, rats received subcutaneous injection of calcitonin or intraperitoneal injections of both calcitonin and calcium (4.0 mg/100. g) The bile collection after hormone administration was done for 90 min. Each point represents the mean of 5 or 6 animals. Vertical lines represent the SE. ○——○; calcitonin, ●——●; calcitonin and calcium.

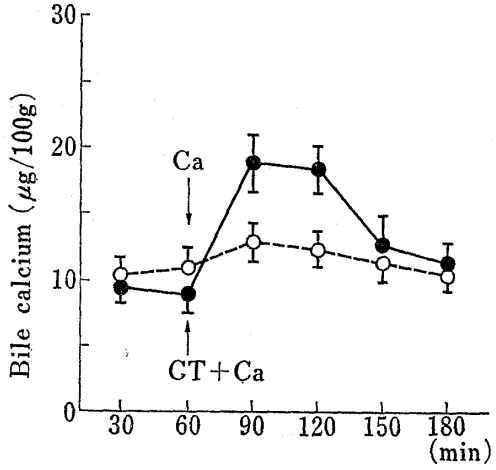

Fig. 3. Time Course of the Increased Calcium Excretion Induced by Calcitonin in Thyroparathyroidectomized Rats

Rats were thyroparathyroidectomized 24 hr prior to the collection of bile. Rats received intraperitoneal injection of calcium (4.0 mg/100 g) or both subcutaneous injection of calcitonin (80 MRC mU/ /100 g) and intraperitoneal injection of calcium (4.0 mg/100 g) 60 min after initiation of bile collection. Each point represents the mean of 5 or 6 animals. Vertical lines represent the SE. ○——○; calcium, ●——●; calcitonin and calcium.

The bile calcium excretion in the presence of increasing concentrations of CT after calcium injection is shown in Fig. 2. The bile calcium concentration after the administration of CT to rats injected with calcium (4.0 mg/100 g) showed approximately 2 times of the values that observed in the hormone alone even at the lowest dose tested (40 MRC mU/100 g). With higher doses, the effect of hormone was slightly greater.

The time course of CT action on the bile calcium excretion of rats injected with calcium is shown in Fig. 3. CT (80 MRC mU/100 g) and calcium (4.0 mg/100 g) administered simultaneously 60 min after the start of bile collection. The results indicate that, as early as 30 min after the administration of hormone to thyroparathyroidectomized rats receiving calcium, there was a remarkable increase in bile calcium excretion. The effect of hormone, however, was temporary since bile calcium dropped to control levels during 90 min after hormone administration. Meanwhile, an injection of calcium alone to thyroparathyroidectomized rats did not increase significantly bile calcium excretion.

Discussion

In the present studies we demonstrated that calcium concentration in the bile of rats markedly increased rapidly after the injection of calcium. This increase depended on the dose of calcium. This suggests that the elevation of serum calcium may induce the excretion of calcium into bile of rats. However, the injection of calcium to thyroparathyroidectomized rats did not cause a significant increase in the bile calcium excretion. This prevention by thyroparathyroidectomy was completely restored by the administration of CT (80 MRC mU/100 g). Accordingly, it is likely that an inhibitory effect of thyroparathyroidectomy on the bile calcium excretion after calcium injection is due to no endogenous release of CT. Presumably, CT promotes the excretion of calcium into the bile of rats.

On the other hand, Tarnawski *et al.*,[5] reported that the decrease in plasma calcium by a single dose of CT (20 MRC U/animal) was not accompanied by any significant changes in the concentration of its component in the bile and that CT causes an increase in calcium concentration in the liver tissue of guinea pigs. The apparent discrepancy between our results and those obtained by them is possibly attributable to differences in the species of animals and the dose of hormone (especially they employed high concentration of CT).

With the lowest dose tested of 40 MRC mU per 100 g body weight, the bile calcium excretion was potentiated about 2 times by CT administration to thyroparathyroidectomized rats receiving calcium when compared with that of the calcium injection alone. This suggests that smaller amount of hormone are effective in eliciting a physiologically important bile response. Also, the effect of CT on the bile calcium excretion occurred during 60 min after hormone administration. Thus CT causes a rapid but temporary increase in the bile calcium excretion.

CT is secreted from the thyroid gland by the elevation of serum calcium and exhibits the hypocalcemic effect.[10] Meanwhile, the elevation of serum calcium causes the augmentation of calcium excretion into the bile.[11] In the present studies, CT stimulated the bile calcium excretion after calcium injection to thyroparathyroidectomized rats. Presumably, the stimulatory effect of CT on the bile calcium excretion may play a part to prevent an increase in serum calcium. It seems reasonable to speculate that the bile pole of the hepatocyte participates in an alteration of calcium metabolism induced by CT.

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