[Chem. Pharm. Bull. 27(7)1671—1674(1979)]

UDC 615.272.015.42.033.034.076.9

Effects of Various Calcitonins on Calcium Concentrations in the Bile and Serum of Thyroparathyroidectomized Rats

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(Received September 7, 1978)

The effects of porcine calcitonin (CT), salmon CT, or synthetic eel CT ([Asu[1,7]] ECT) on the calcium concentrations in the bile and serum have been examined after a single intraperitoneal administration of calcium chloride to thyroparathyroidectomized rats. The administration of these hormones (80 MRC mU/100 g body weight, respectively) markedly increased calcium excretion into the bile within 1 hr after the injection of calcium chloride (4.0 mg Ca/100 g). The effect of synthetic eel CT was more prolonged than that of any other hormone (up to 3 hr after administration). The administration of porcine CT, salmon CT, or synthetic eel CT significantly inhibited the elevation of serum calcium concentration within 1 hr after the injection of calcium. The serum calcium concentration was slightly decreased at 3 hr after the administration of salmon CT or synthetic eel CT. These results suggest that the lowering effect of CT on the serum calcium level results partly from the stimulation of calcium excretion into the bile.

Keywords——calcitonin; calcium; stimulatory effect on bile calcium excretion; hypocalcemic effect of calcitonin; thyroparathyroidectomized rats

Introduction

It is well known that calcitonin (CT) has a hypocalcemic effect. This effect of CT is reportedly due to the inhibition of bone absorption,[2)] the reduction of intestinal calcium absorption,[3)] and enhanced urinary calcium excretion.[4)] Recentry it has been reported that calcium excretion into the bile is increased by the elevation of serum calcium,[5)] and that CT markedly increased calcium excretion into the bile of thyroparathyroidectomized rats.[6)] On the basis of these results, it has been suggested that the stimulatory effect of CT on calcium excretion into the bile may play a part in preventing an increase in serum calcium.[6)] The present study was therefore undertaken to examine whether the hypocalcemic effect of CT involves the hormonal augmentation of calcium excretion into the bile. This paper describes the effects of porcine CT, salmon CT, and synthetic eel CT on the calcium concentrations in the serum and bile of thyroparathyroidectomized rats.

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 and Hormones——Calcium chloride was dissolved in sterile, demineralized water. Porcine and salmon calcitonins were purchased from Armour Pharmaceutical Company (68 MRC U/mg, and 200 MRC U/mg, respectively, Kankakee, Ill., U.S.A.). Synthetic eel calcitonin ([Aus[1,7]] ECT) was supplied through the courtesy of Toyo Jozo Research Laboratories (4000 MRC U/mg, Shizuoka, Japan). These hormones were dissolved in sterile, demineralized water. The vehicle was injected as a control.

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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 body weight) 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±1°) to maintain the body temperature,[7] and the bile was collected. The rats were not fed or given water. The administration of calcium chloride (4.0 mg Ca/0.5 ml/100 g) was carried out intraperitoneally at the midpoint of the abdomen. Immediately after the administration of calcium, the hormone (0.5 ml/100 g) was administered subcutaneously at a separate site.

Analytical Methods——The bile was collected three times at one hour intervals after the administration of hormone. The bile volume was measured by means of pipet graduated in 0.01 ml. The amount of calcium was determined by atomic absorption spectrophotometry (Perkin-Elmer, model 303) with a reverse air-acetylene flame after precipitation with 10% trichloroacetic acid.[8] The bile calcium concentration was expressed in two ways: (i) amount of bile calcium, defined as the excreted calcium (μg) per 100 g body weight of the rat; and (ii) content of bile calcium, defined as calcium (μg) per milliliter of bile.

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 analyzed immediately. The calcium content was determined using 0.1 ml aliquots of serum by atomic absorption spectrophotometry.[8]

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.

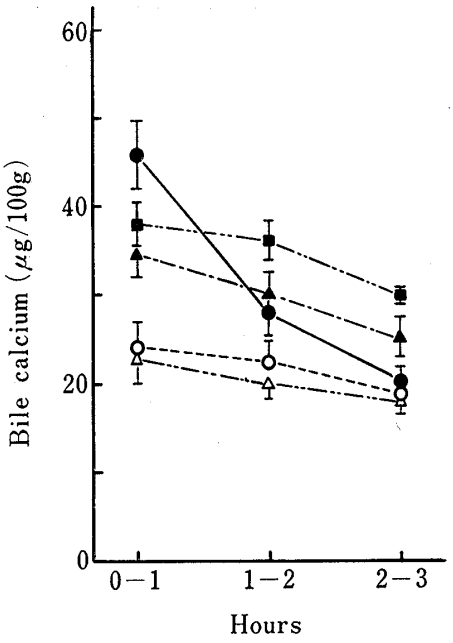

Fig. 1. Time Course of Calcium Excretion into the Bile After the Administration of Porcine Calcitonin (CT), Salmon CT, or Synthetic Eel CT to Thyroparathyroidectomized Rats Injected with Calcium Chloride

Rats were thyroparathyroidectomized 24 hr before the collection of bile. Rats received an intraperitoneal injection of calcium (4.0 mg/100 g) or both a subcutaneous administration of porcine CT, salmon CT, or synthetic eel CT (80 MRC mU/100 g, respectively) and an intraperitoneal injection of calcium (4.0 mg/100 g). Each point represents the mean of 5 or 6 animals. Vertical lines represent the SE. ----△----, None; ----○----, calcium (Ca); ——●——, porcine CT and Ca; ----▲----, salmon CT and Ca: ----■----, synthetic eel CT and Ca.

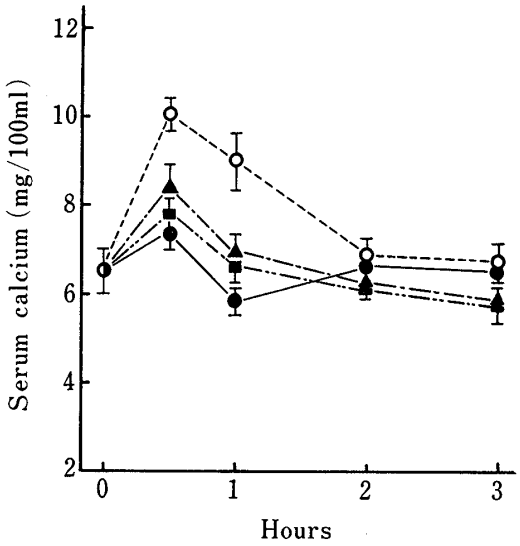

Fig. 2. Time Course of Calcium Concentration in the Serum After the Administration of Porcine Calcitonin (CT), Salmon CT, or Synthetic Eel CT to Thyroparathyroidectomized Rats Injected with Calcium Chloride

Rats were thyroparathyroidectomized 24 hr before the experiments. Rats received an intraperitoneal injection of calcium (4.0 mg/100 g) or both a subcutaneous administration of porcine CT, salmon CT, or synthetic eel CT (80 MRC mU/100 g, respectively) and an intraperitoneal injection of calcium (4.0 mg/100 g). Each point represents the mean of 5 or 6 animals. Vertical lines represent the SE. ----○----, calcium (Ca); ——●——, porcine CT and Ca; ----▲----, salmon CT and Ca; ----■----, synthetic eel CT and Ca.

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Results

The time course of CT action on the excretion of calcium into the bile of thyroparathyroidectomized rats is shown in Fig. 1. The calcium content in the bile of the control rats gradually decreased during the collection period. When the solution of calcium chloride (4.0 mg Ca/100 g) was administered intraperitoneally, the calcium content in the bile did not increase significantly in comparison with that of the control rats. Meanwhile, porcine CT, salmon CT, or synthetic eel CT (80 MRC mU/100 g, respectively) was subcutaneously administered immediately after a single intraperitoneal injection of calcium chloride (4.0 mg Ca/100 g) to thyroparathyroidectomized rats. The results indicate that, as early as 1 hr after the administration of each hormone, there was a significant increase in calcium excretion into the bile. Porcine CT produced a marked elevation of calcium excretion into the bile within 1 hr after administration of the hormone, while salmon CT caused a significant increase in the bile calcium excretion within 2 hr. Synthetic eel CT significantly increased the calcium excretion into the bile even at 3 hr after administration of the hormone. This effect of synthetic eel CT was more long-lasting than that of any other hormone used in these experiments.

The total amount of calcium excreted into the bile during 3 hr is summarized in Table I. The administration of porcine CT, salmon CT, or synthetic eel CT did not significantly change the bile volume, while these hormones markedly increased the content of calcium in the bile.

Table I. Effects of Porcine Calcitonin (CT), Salmon CT, or Synthetic Eel CT on Calcium Excretion into the Bile of Thyroparathyroidectomized Rats Injected with Calcium Chloride

Treatment[a]	Number of rats	Bile volume[b] (ml/100 g)	Bile calcium concentration[b]	
			(μg/100 g)	(μg/ml)
None	5	1.28±0.16	60.3±6.9	48.5±3.9
Calcium (Ca)	6	1.21±0.18	68.8±6.9	56.9±4.8
Ca+porcine CT	5	1.24±0.10	95.0±6.3[c]	76.6±3.1[c]
Ca+salmon CT	5	1.19±0.14	89.5±5.5[c]	73.5±7.0[c]
Ca+synthetic eel CT	6	1.19±0.07	101.2±3.0[c]	86.0±7.4[c]

a) Calcium (4.0 mg/100 g) was injected intraperitoneally 24 hr after thyroparathyroidectomy, and porcine CT, salmon CT, or synthetic eel CT (80 MRC mU/100 g, respectively) was immediately administered subcutaneously. The bile was collected for 3 hr after administration of the hormone.
b) Values are mean±SEM.
c) Significance of the difference from the calcium mean, $p < 0.01$ (Student's *t*-test).

On the other hand, the effects of porcine CT, salmon CT, or synthetic eel CT on the serum calcium concentration after a single intraperitoneal administration of calcium to thyroparathyroidectomized rats is shown in Fig. 2. The animals were killed at various periods after administration of the hormone. The calcium concentration in the serum reached a maximum ($p<0.01$) 30 min after the administration of calcium chloride (4.0 mg Ca/100 g), and then decreased rapidly. When porcine CT, salmon CT, or synthetic eel CT (80 MRC mU/100 g) was subcutaneously administered immediately after the injection of calcium chloride (4.0 mg Ca/100 g), these hormones significantly inhibited the increase of serum calcium concentration within 1 hr after the administration of calcium. The calcium concentration in the serum at 2 hr after the administration of synthetic eel CT was significantly lower than the values obtained from rats injected with porcine CT. At 3 hr after the administration of salmon CT or synthetic eel CT, the calcium concentration in the serum was slightly decreased but the effect was not significant.

Discussion

In the present study, it was found that porcine CT, salmon CT, and synthetic eel CT, which have a hypocalcemic effect, stimulated calcium excretion into the bile after a single intraperitoneal administration of calcium chloride to thyroparathyroidectomized rats. Porcine CT or salmon CT produced an increase in bile calcium within 1 hr or 2 hr, respectively, after administration. In particular, synthetic eel CT significantly increased bile calcium even at 3 hr after administration of the hormone. Thus the stimulatory effect of synthetic eel CT on calcium excretion into the bile was more prolonged than that of porcine CT or salmon CT. This may be because the biological half-life of synthetic eel CT in the blood is longer than that of porcine CT or salmon CT.[9]

The administration of porcine CT, salmon CT, or synthetic eel CT produced a remarkable increase in bile calcium and a corresponding fall in serum calcium within 1 hr after the injection of calcium into thyroparathyroidectomized rats. It is established that the hypocalcemic effect of CT is due to the inhibition of calcium mobilization from the bone,[2] the reduction of calcium transport in the intestine,[3] and enhanced calcium excretion into the urine.[4] Based on the present results, however, it is possible that the prompt lowering effect of CT on serum calcium results partly from the stimulation of calcium excretion into the bile produced by the hormone.

Recently we reported that the calcium concentration in the serum is markedly enhanced by ligation of the bile duct in rats orally administered with calcium chloride,[10] suggesting that calcium excretion into the bile prevents the elevation of calcium concentration in the serum after calcium absorption from the intestine. The bile pole of the hepatocytes presumably participates in the regulation of calcium level in the serum, and CT presumably plays a physiological role in the control of calcium metabolism in the bile hepatic system.

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