INTERACTION OF CALCITONIN AND HISTAMINE ON BILE CALCIUM EXCRETION IN THYROPARATHYROIDECTOMIZED RATS

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The effects of calcitonin (CT) and histamine on calcium metabolism in the hepatic bile system of rats were investigated. The subcutaneous administration of CT (20 and 80 MRC mU/100 g) to rats caused a significant fall in serum calcium and a marked increase in liver calcium. The subcutaneous administration of histamine (0.1 mg/100 g) to rats produced a significant decrease in serum calcium, while it did not significantly alter liver calcium. However, the effects of CT (20 or 80 MRC mU/100 g) on the serum and liver calcium was markedly prevented by the simultaneous administration of histamine (0.1 mg/100 g). On the other hand, the excretion of calcium into the bile of thyroparathyroidectomized rats intraperitoneally injected with the calcium chloride (4.0 mg Ca/100 g) was markedly increased by the administration of CT (20 or 80 MRC mU/100 g) or histamine (0.1 mg/100 g). Reversely, a remarkable elevation of bile calcium by CT was clearly prevented by the simultaneous administration of histamine (0.1 mg/100 g). The present results further support the point of view that the hypocalcemic effect of CT is based on the stimulation of calcium excretion into the bile by the hormone.

Keywords—calcitonin; histamine; calcium; serum; liver; bile; calcium metabolism

Calcitonin (CT), a calcium-regulating hormone, has a hypocalcemic effect. This effect of CT is reportedly due to the inhibition of bone absorption,¹⁾ the reduction of intestinal calcium absorption,²⁾ and the enhancement of urinary calcium excretion.³⁾ Previously, it has been found that CT increases the concentration of calcium in the liver of rats.⁴⁾ Based on this findings, it has been demonstrated that the liver participates in the regulation of calcium metabolism by CT.

Recently, we have reported that CT markedly increases the excretion of calcium into the bile of thyroparathyroidectomized rats,⁵⁾ suggesting that the bile calcium excretion regulates the levels of calcium in the serum.⁶⁾ On the other hand, it has been recently known that histamine has a hypocalcemic effect in rats.⁷⁾ The present study was therefore undertaken to investigate an interaction of CT and histamine on the bile calcium excretion in 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 Hormone—Calcium chloride was dissolved in sterile, demineralized water. Porcine calcitonin was purchased from Armour Pharmaceutical company (68 MRC U/mg, Kankakee, Ill., U.S.A.). The hormone was dissolved in sterile, demineralized water. Histamine (Wako Pure Chemical Industries, Ltd., Osaka) was dissolved in sterile, demineralized water. 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 ($38 \pm 1^\circ$) to maintain the body temperature, and the bile was collected. The rats were not fed or given water. The administration of calcium chloride (4.0 mg Ca/ml/100 g) was carried out intraperitoneally at the midpoint of the abdomen. Immediately after the administration of calcium, CT or histamine (0.5 ml/100 g, respectively) was subcutaneously administered at a separate site.

Analytical Methods — The animals were bled by cardiac puncture under light anesthesia with ether. Blood samples obtained by cardiac puncture were immediately centrifuged after collection. The serum was separated and analyzed immediately. The calcium contents was determined by atomic absorption spectrophotometry after precipitation with 10% trichloroacetic acid.⁸⁾

Liver was perfused with cold 0.25 M sucrose solution after bleeding and removed immediately. The amount of calcium was determined by atomic absorption spectrophotometry after the digestion with nitric acid.

Bile was collected for 90 min after the administration of CT or histamine. The bile volume was measured by means of pipet gradu-

ated in 0.01 ml. The amount of calcium was determined by atomic absorption spectrophotometry after precipitation with 10% trichloroacetic acid. 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.

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 difference between values was estimated by Student's *t* test. *p* values less than 0.05 were considered to indicate statistically significant differences.

RESULTS

Effects of Calcitonin and Histamine on Serum and Liver Calcium

The effects of calcitonin (CT) and histamine on the serum calcium in rats are shown in Table I. The administration of CT (20 or 80 MRC mU/100 g body weight) produced a significant reduction of serum calcium at 30 and 60 min after the dosing of hormone. The administration of histamine (0.1 mg/100 g) caused a significant decrease of serum calcium at 30 min after the application of drug. Conversely, the serum

TABLE I. *Effects of Calcitonin (CT) and Histamine on Serum Calcium in Rats*

Treatment ^{a)}	Serum calcium (mg/100 ml) ^{b)}	
	30 min	60 min
Control	10.28 $\pm$ 0.10	10.11 $\pm$ 0.18
CT (20 MRC mU/100 g)	9.11 $\pm$ 0.15 ^{c)}	9.01 $\pm$ 0.18 ^{c)}
CT (80 MRC mU/100 g)	8.31 $\pm$ 0.14 ^{c)}	8.02 $\pm$ 0.11 ^{c)}
Histamine	9.63 $\pm$ 0.04 ^{c)}	10.20 $\pm$ 0.24
CT (20 MRC mU/100 g) + histamine	9.88 $\pm$ 0.16 ^{d)}	9.63 $\pm$ 0.20
CT (80 MRC mU/100 g) + histamine	9.41 $\pm$ 0.08 ^{c,d)}	9.34 $\pm$ 0.16 ^{c,d)}

a) The rats were subcutaneously administered simultaneously CT (20 or 80 MRC mU/100g) and histamine (0.1 mg/100 g).

b) Values are mean $\pm$ SE for 5 animals.

c) *p* < 0.01 as compared with control.

d) *p* < 0.01 as compared with CT (80 MRC mU/100 g).

TABLE II. *Effects of Calcitonin (CT) and Histamine on Liver Calcium in Rats*

Treatment ^{a)}	Liver calcium ($\mu\text{g/g}$ wet tissue) ^{b)}	
	30 min	60 min
Control	13.0 $\pm$ 1.02	12.2 $\pm$ 1.31
CT (20 MRC mU/100 g)	18.0 $\pm$ 1.21 ^{c)}	18.4 $\pm$ 1.36 ^{c)}
CT (80 MRC mU/100 g)	20.0 $\pm$ 1.82 ^{c)}	21.2 $\pm$ 1.94 ^{c)}
Histamine	11.8 $\pm$ 0.76	12.2 $\pm$ 1.70
CT (20 MRC mU/100 g) + histamine	10.3 $\pm$ 0.51 ^{d)}	10.2 $\pm$ 0.45 ^{d)}
CT (80 MRC mU/100 g) + histamine	10.6 $\pm$ 0.94 ^{d)}	12.2 $\pm$ 0.24 ^{d)}

a) The rats were subcutaneously administered simultaneously CT (20 or 80 MRC mU/100g) and histamine (0.1 mg/100 g).

b) Values are mean $\pm$ SE for 5 animals.

c) $p < 0.01$ as compared with control.

d) $p < 0.01$ as compared with CT (20 or 80 MRC mU/100 g).

calcium was not significantly decreased by the simultaneous administration of CT (20 MRC mU/100 g) and histamine (0.1 mg/100 g) in comparison with that of the control rats. Moreover, the lowering effect of CT on serum calcium was markedly prevented by the simultaneous administration of CT (80 MRC mU/100 g) and histamine (0.1 mg/100 g).

The effects of CT and histamine on liver calcium in rats are shown in Table II. The liver calcium was markedly increased by the administration of CT (20 or 80 MRC mU/100 g), while it was not significantly altered by histamine (0.1 mg/100 g). The simultaneous administration of CT (20 or 80 MRC mU/100 g) and histamine (0.1 mg/100 g) did not cause any significant elevation of liver calcium in comparison with that of the control rats.

Effect of Histamine on Bile Calcium Excretion

The time course of histamine action on the excretion of calcium into the bile of thyroparathyroidectomized rats is shown in Fig. 1. The calcium content in the bile after intraperitoneal injection of calcium chloride (4.0 mg Ca/100g) did not significantly increase in comparison with that of the control rats. Meanwhile, histamine (0.1 mg/100 g) was subcutaneously administered immediately after a single intraperitoneal injection of calcium chloride (4.0 mg Ca/100 g). The

results indicate that, as early as 30 min after the administration of histamine, there was a significant increase in calcium excretion into the bile. This augmentation was observed even at 60 min after the administration of the drug.

FIG. 1. *Time Course of Histamine Effect on Bile Calcium Excretion in Thyroparathyroidectomized Rats*

The rats were thyroparathyroidectomized 24 hr prior to bile collection, and they were subcutaneously administered histamine (0.1 mg/100 g) immediately after intraperitoneal injection of calcium (4.0 mg/100 g). Each bar represents the mean of 5 animals. The vertical lines give the SE.

□; Control, ▨; calcium, ■; calcium + histamine.

The effects of increasing doses of histamine on the excretion of calcium into the bile of thyroparathyroidectomized rats are shown in Fig. 2. The bile was collected for 90 min after the

FIG. 2. Dose Effect of Histamine on Bile Calcium Excretion in Thyroparathyroidectomized Rats

The rats were thyroparathyroidectomized 24 hr prior to bile collection, and they were subcutaneously administered histamine immediately after intraperitoneal injection of calcium (4.0 mg/100 g). The bile was collected for 90 min. Each bar represents the mean of 5 animals. The vertical line give the SE.

administration of histamine. The bile calcium after the administration of histamine to thyroparathyroidectomized rats injected with calcium chloride (4.0 mg Ca/100 g) showed a significant elevation even at the lowest dose tested (0.01 mg/100 g). With the higher doses, the effect was greater, but the differences between the two higher doses (0.1 and 1.0 mg/100 g) was not significant.

Interaction of CT and Histamine on Bile Calcium Excretion

An interaction of CT and histamine on the excretion of calcium into the bile of thyroparathyroidectomized rats is shown in Table III. A significant augmentation of bile calcium by CT was observed within 90 min after the administration of the hormone.⁵⁾ Then, the bile was collected for 90 min following the administration of CT or histamine. CT or histamine was subcutaneously administered to thyroparathyroidectomized rats receiving calcium (4.0 mg/100 g) intraperitoneally. The administration of CT (20 or 80 MRC mU/100 g) or histamine (0.1 mg/100 g) did not cause a significant alteration of bile volume. The bile calcium was significantly increased by the administration of CT (20 or 80 MRC mU/100 g) and histamine (0.1 mg/100 g). However,

TABLE III. Effects of Calcitonin (CT) and Histamine on Bile Calcium Excretion in Thyroparathyroidectomized Rats

Treatment ^{a)}	Bile volume ^{b)} (ml/100 g)	Bile calcium ^{b)}	
		(μg/100 g)	(μg/ml)
Control	0.61 ± 0.04	41.0 ± 4.4	65.6 ± 6.2
CT (20 MRC mU/100 g)	0.65 ± 0.09	68.0 ± 12.6 ^{c)}	101.6 ± 7.2 ^{c)}
CT (80 MRC mU/100 g)	0.62 ± 0.04	83.3 ± 5.2 ^{c)}	134.8 ± 10.8 ^{c)}
Histamine	0.60 ± 0.02	64.0 ± 3.8 ^{c)}	105.6 ± 9.0 ^{c)}
CT (20 MRC mU/100 g) + histamine	0.64 ± 0.03	49.7 ± 3.5 ^{d)}	78.3 ± 4.5 ^{d)}
CT (80 MRC mU/100 g) + histamine	0.68 ± 0.07	62.1 ± 5.9 ^{c,d)}	93.6 ± 7.6 ^{c,d)}

a) The rats were thyroparathyroidectomized 24 hr prior to bile collection, and they were subcutaneously administered simultaneously CT (20 or 80 MRC mU/100 g) and histamine (0.1 mg/100 g) immediately after intraperitoneal injection of calcium (4.0 mg/100 g). The bile was collected for 90 min.

b) Values are mean ± SE for 5 animals.

c) $p < 0.01$ as compared with control.

d) $p < 0.01$ as compared with CT (20 or 80 MRC mU/100 g).

histamine markedly prevented the elevation of bile calcium after the administration of CT, while the bile volume did not change significantly.

DISCUSSION

It is well known that calcitonin (CT) has a hypocalcemic effect. From the previous investigations,^{5,6)} it is suggested that CT plays a part in the regulation of calcium level in the serum due to promotion of calcium excretion into the bile of rats. On the other hand, it has been reported that histamine has a hypocalcemic effect in rats.⁷⁾ This action is related to acid secretion in the stomach and not to the thyroid release of CT in rats.⁹⁾

The present study demonstrates that the administration of histamine produced a fall in serum calcium of rats, and that it caused a marked increase in calcium excretion into the bile of thyroparathyroidectomized rats. From this results, it is assumed that the hypocalcemic effect of histamine partly results from the stimulation of calcium excretion into the bile by the drug.

The simultaneous administration of histamine to rats treated with CT caused clear prevention of both a fall in serum calcium and a raise in liver calcium due to CT. Additionally a remarkable enhancement of calcium excretion into the bile of thyroparathyroidectomized rats treated with CT was significantly inhibited by the simultaneous administration of histamine. Such an inhibition of histamine on the augmentation of both liver and bile calcium due to CT may induce a retardation of the lowering effect of CT on serum calcium, since CT increases the calcium in the liver by stimulating uptake of the calcium from the serum,⁴⁾ and thus causes a remarkable elevation of calcium excretion into the bile.⁵⁾

Presently we do not know the mechanisms by which histamine inhibits calcium accumulation in the liver caused by CT. It is possible, however, that histamine antagonizes binding of CT to plasma membrane, since CT increases the concentration of calcium in the liver cells by inhibit-

ing extracellular efflux of intracellular calcium.¹⁰⁾

In conclusion, the results obtained from the present investigation further support a point of view that the revelation of the hypocalcemic effect of CT results from the promotion of calcium excretion into the bile based on an increase in liver calcium due to the hormone. CT plays an important role in the control of calcium homeostasis in the serum by regulating calcium metabolism in the hepatic bile system of the rats.

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