[Chem. Pharm. Bull. 25(9)2189—2194(1977)]

UDC 615.273.015.45.076.9 : 547.457.1.08

Effect of Calcitonin on Serum Glucose Concentration in Rats

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(Received October 14, 1976)

Calcitonin (CT) administered subcutaneously to intact and thyroparathyroidectomized rats caused a significant decrease in the serum calcium, while the serum glucose concentration increased. The maximal response was obtained with 80 MRC mU/100 g body weight. The lowest dose of CT which produces a detectable effect was approximately 20 MRC mU. Glucagon (5 μg/100 g) caused a rapid elevation in the serum glucose, reaching a maximum level within 15 min, whereas CT caused a more gradual increase in the serum glucose, reaching a maximum after 60 min. Administration of CT to fasted rats caused a significant increase in the serum glucose. Liver glycogen levels were relatively decreased by CT administration. Administration of CT produced the accumulation of calcium in liver cells and significantly elevated glucose-6-phosphatase and phosphorylase activities in liver homogenate. These results suggest that a rise in the serum glucose concentration produced by CT may involve the production of glucose in the liver.

Keywords——calcitonin; serum glucose; hyperglycemic effect of calcitonin; glycogenolysis by calcitonin; rats

Introduction

Calcitonin (CT) has a hypocalcemic effect[2] which is reportedly attributed to the inhibition of calcium release from the bone by a hormone.[2–4] Recently, it is known that CT inhibited glucose uptake stimulated by insulin in diaphragm muscle of rats, and that the effect of CT is not mediated by hypocalcemia.[5,6] Ziegler, *et al.* have found that CT provoked a significant impairment of glucose assimilation and insulin output in man.[7] Thus it suggests that CT has an insulin inhibitory effect not mediated through hypocalcemia.

In more recent studies, CT has been shown to increase the calcium concentration in the livers of intact and thyroparathyroidectomized rats.[8] It is assumed that an increase in hepatic calcium concentration following CT administration affects the mertabolic system in liver cells. The uptake of calcium by liver cells is related to the regulation of glycolysis and gluconeogenesis.[9] CT may cause the production of glucose in the liver. The present study was therefore undertaken to examine the effect of CT on glucose concentration in the serum of rats. We found that CT increases glucose concentration in the serum.

Materials and Methods

Animals——Male Wistar rats, weighing approximately 120 g, were used in this experiment. They were obtained commercially (Nippon Bio Supp. Center Co., Ltd., Tokyo). The animals were fed commercial lab. chow (containing 7.4% carbohydrate, 1.1% Ca and 1.1% P, Oriental Test Diet Co., Ltd., Tokyo) and tap water freely.

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Hormones——Calcium chloride and EDTA were dissolved in sterile, demineralized water. Calcitonin (lyophilized porcine or salmon calcitonin, 68 MRC U/mg or 200 MRC U/mg respectively, Armour Pharmaceutical Company, Kankakee, Ill.) was dissolved in sterile, demineralized water. Glucagon (supplied through the courtesy of Eli Lilly Research Laboratories, Indianapolis, Ind.) was dissolved in sterile demineralized water. Control injections consisted of sterile, demineralized water.

Surgical Procedures——Surgical procedures were done under light ether anesthesia. The thyroparathyroid gland complex was removed with fine forceps.

Analytical Methods——Blood was obtained by cardiac puncture and centrifuged immediately. The amount of calcium in the serum was determined by atomic absorption spectrophotometer (Perkin-Elmer, Model 303) with a reversed air-acetylene flame after precipitation with 10% trichloroacetic acid.[10] Serum glucose levels were measured with the Glytel *o*-toluidine reagent.[11] Liver was removed immediately after bleeding and rinsed with an ice-cold 0.9% NaCl solution. Liver glycogen was determined by a modification of the method of Good, *et al.*[12] The tissues were digested in 30% KOH in a boiling water bath for 1 hr and glycogen was precipitated by adding 95% ethanol and allowing the samples to stand overnight.[13] The samples were then centrifuged and the supernatant was decanted. Glycogen was then washed with ethanol, hydrolyzed with 1 M H_2SO_4, diluted with distilled water, and aliquots were analyzed for glucose by using the Glytel reagent. The weight of glycogen is expressed in terms of milligrams of glucose.

Enzyme Assays——Glucose-6-phosphatase activity[14] was determined by incubating a portion of the homogenate from a piece of fresh liver homogenized in distilled water in a system containing 40 mM glucose 6-phosphate, 7 mM histidine, and 1 mM EDTA, pH 6.5, for 20 min at 37°. The release of inorganic phosphate was measured according to the method of Nakamura.[15] In this procedure a linear relationship exists between the time and the amount of homogenate assayed. The glucose-6-phosphatase activity is expressed as n mol of inorganic phosphate released per min per mg protein.

The assay of phosphorylase activity involved the incorporation of glucose 1-phosphate into a primer of glycogen with the release of inorganic phosphate. The reaction mixture contained 0.1 ml of the homogenate, glucose 1-phosphate (7.5 mg/ml), and glycogen (3 mg/ml) in a final volume of 1 ml.[16] This mixture was incubated at 38° for 10 min and 1 ml of 5% trichloroacetic acid was added. After centrifugation, 1 ml of the supernatant was taken for the determination of inorganic phosphate.[15] The phosphorylase activity is expressed as n mol inorganic phosphate released per min per mg protein. The homogenate protein concentration was determined according to the method of Lowry, *et al.*[17] with bovine serum albumin as a standard.

Statistical Methods——The data were subjected to an analysis of variance, and standard error (SE) was calculated from the residual error term. Statistical significance is expressed as P values from Student's *t*-test.

Results

Effect of CT on Serum Glucose Concentration

In the first experiment, to investigate the time course of calcitonin (CT) action on serum glucose and calcium, CT (80 MRC mU/100 g) was administered subcutaneously to intact rats and their blood was obtained at varying periods after hormone injection. Figure 1 shows that, as early as 15 min after hormone administration, there was an increase in serum gluscoe, while a significant decrease in serum calcium concentration appeared. The serum glucose concentration continued to increase, reaching a maximum at 60 min, and returned to normal levels 90 min after hormone injection. The effect of increasing doses of CT on the serum glucose is shown in Fig. 2. The animals were killed 1 hr after hormone administration. The hormone significantly increased the serum glucose even at the lowest dose (20 MRC mU/100 g), and there was the corresponding fall in serum calcium. With higher doses, the effect was greater, but the effect of 160 MRC mU of CT was significantly less than that of 80 MRC mU.

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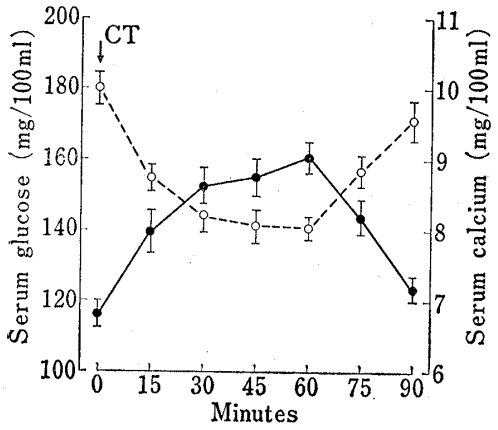

Fig. 1. Time Course of Serum Glucose and Calcium After Administration of Calcitionin (CT) to Rats

CT (80 MRC mU/100 g) was administered subcutaneously. Each point represents the mean of 5 or 6 animals. Vertical lines represent the SE.
●——●, serum glucose; ○------○, serum calcium.

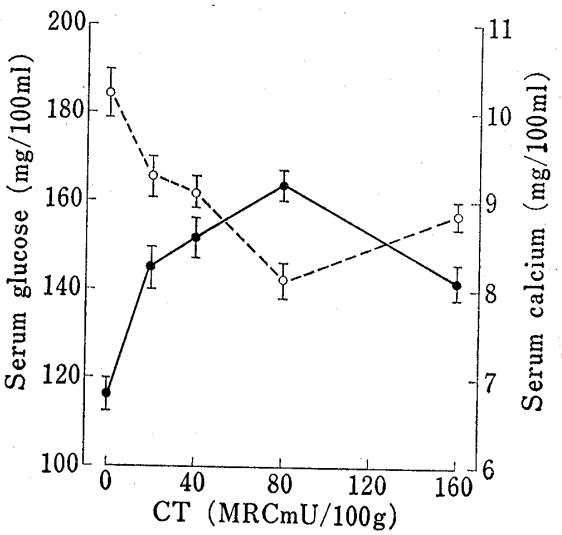

Fig. 2. Effect of Calcitonin (CT) on the Serum Glucose and Calcium of Rats

CT (80 MRC mU/100 g) was administered subcutaneously, and rats were bled 1 hr after CT. Each point represents the mean of 5 or 6 animals. Vertical lines represent the SE.
●——●, serum glucose; ○------○, serum calcium.

The effect of salmon CT on the serum glucose was compared with that of porcine CT. The results indicate that salmon CT (80 MRC mU/100 g) significantly increased the serum glucose concentration of rats, but the effect of salmon CT was significantly less than that of porcine CT (Table I).

TABLE I. Effect of Porcine and Salmon Calcitonin (CT) on the Serum Glucose and Calcium of Rats

Treatment	CT[a] (MRC mU/100 g)	Number of rats	Serum glucose (mg/100 ml)	Serum calcium (mg/100 ml)
Control[b]		6	114.5±2.9[c]	10.2 ±0.31
Porcine CT	40	5	147.0±5.2[d]	9.15±0.02[d]
	80	5	165.1±2.6[d]	8.10±0.13[d]
Salmon CT	40	5	118.1±3.4	8.56±0.20[d]
	80	6	133.5±2.7[d,e]	8.07±0.40[d]

a) CT was administered subcutaneously and rats bled 1 hr after CT injection.
b) Control injected sterile distilled water.
c) Eeach value represents the mean ± S.E.
d) Differs fom respective control mean, $p<0.01$.
e) Differs from respective porcine CT (80 MRC mU/100 g).

TABLE II. Effect of Porcine Calcitonin (CT) on the Serum Glucose and Calcium of Thyroparathyroidectomized Rats

Treatment	Number of rats	Serum glucose (mg/100 ml)	Serum calcium (mg/100 ml)
Control[a]	6	132.1±4.2[b]	6.51±0.37
CT[c]	7	162.0±3.9[d]	5.52±0.20[d]

a) Control injected sterile distilled water.
b) Each value represents the mean ± S.E.
c) CT (80 MRC mU/100 g) was administered subcutaneously 23 hr after thyroparathyroidectomy. The rats were bled 1 hr after CT injection.
d) Differs from respective control mean, $p<0.01$.

The effect of porcine CT (80 MRC mU/100 g) was studied in rats thyroparathyroidectomized 24 hr before the experiment. The results indicate that CT significantly increased the serum glucose in thyroparathyroidectomized rats (Table II). The administration of CT to thyroparathyroidectomized rats was followed by a fall in serum calcium.

The effect of EDTA (30 mg/100 g) on the serum glucose concentration was studied in rats to determine whether hypocalcemia itself causes an increase in the serum glucose concentration. The animals were killed 1 hr after a single intraperitoneal administration of EDTA. Administration of EDTA to rats did not significantly increase their serum glucose concentration, but had the same effect on the serum calcium as CT administration (Table III).

Table III. Effect of Calcitonin (CT) or EDTA on the Serum Glucose and Calcium of Rats

Treatment	Number of rats	Serum glucose (mg/100 ml)	Serum calcium (mg/100 ml)
Control[a)]	6	121.8±4.6[b)]	9.87±0.21
CT[c)]	5	170.3±4.3[d)]	8.21±0.26[e)]
EDTA	6	133.6±4.5	8.67±0.21[e)]

a) Control injected sterile distilled water.
b) Each value represents the mean ± S.E.
c) CT (80 MRC mU/100 g) was administered subcutaneously.
d) Differs from respective control mean, $p<0.01$.
e) EDTA (30 mg/100 g) was administered intraperitoneally. The rats were bled 1 hr after CT or EDTA injection.

The effect of CT (80 MRC mU/100 g) on the serum glucose concentration of rats fasted 24 hr before the hormone injection is shown in Table IV. The animals were killed 1 hr after the hormone. The results showed that CT significantly increased the serum glucose concentration of fasted rats, while it significantly decreased the serum calcium concentration.

Table IV. Effect of Calcitonin (CT) on the Serum Glucose Concentration of Fasted Rats

Treatment	Number of rats	Serum glucose (mg/100 ml)	Serum calcium (mg/100 ml)
Control[a)]	6	67.0±4.0[b)]	6.82±0.04
CT[c)]	7	88.9±2.1[d)]	6.36±0.12[e)]

a) Control injected sterile distilled water.
b) Each value represents the mean ± S.E.
c) CT (80 MRC mU/100 g) was administered subcutaneously.
d) Differs from respective control mean, $p<0.01$.
e) Differs from respective control mean, $p<0.05$.

Effect of Glucagon on Serum Glucose increased by CT

It is known that glucagon affects the concentration of glucose in the serum of rats. To investigate the effect of glucagon on serum glucose increased by CT, glucagon (5 μg/100 g) was administered intraperitoneally to rats receiving CT (80 MRC mU/100 g). The time course of an increase in the serum glucose after a single administration of glucagon or CT is shown in Fig. 3. The animals were killed at various time intervals after hormone administration. The serum glucose concentration increased rapidly ($p<0.01$) by 15 min and then began to decrease 30 min after glucagon treatment. An entirely different pattern was observed for the serum glucose after CT administration. The serum glucose concentration after CT injection increased significantly and reached a maximum level 60 min after hormone treatment. When glucagon was administered immediately after CT injection, the serum glucose concentration increased rapidly and still elevated even after 30 or 60 min.

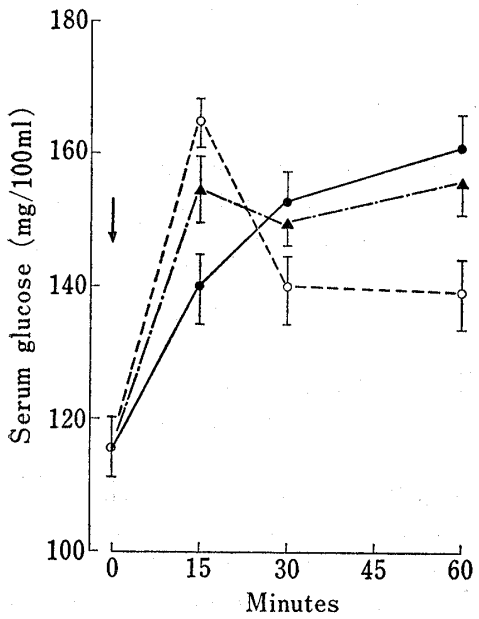

Fig. 3. Effect of Calcitonin (CT) and Glucagon on the Serum Glucose of Rats

The rats received subcutaneously injection ot CT (80 MRC mU/100 g) or intraperitoneal injection of glucagon (5 μg/100 g). Each point represents the mean of 5 or 6 animals. Vertical lines represent the S.E.
●——●, CT; ○------○, glucagon; ▲——▲, CT and glucagon.

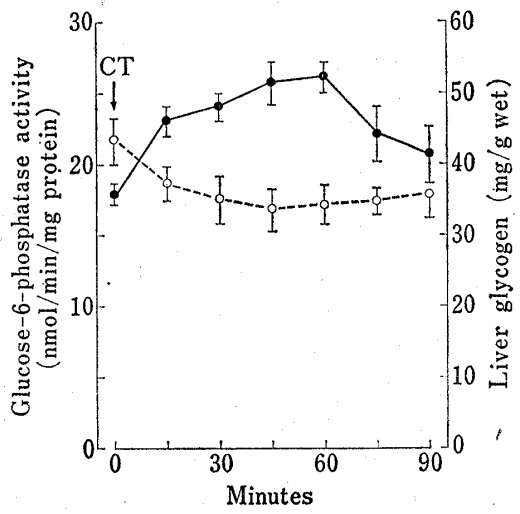

Fig. 4. Effect of Calcitonin (CT) on the Liver Glucose-6-Phosphatase Activity and Glycogen of Rats.

CT (80 MRC mU/100 g) was administered subcutaneously. Each point represents the mean of 5 or 6 animals. Vertical lines represent the S.E.
●——●, glucose-6-phosphatase; ○------○, glycogen.

Effect of CT on Liver Glycogen or Enzyme Activities

The effect of CT (80 MRC mU/100 g) on liver glycogen level was studied in rats. Livers were excised at varying periods after hormone injection. A significant ($p<0.05$) decrease in liver glycogen level was observed at 45 min after hormone administration, and this reduction was still continued even after 90 min (Fig. 4).

CT administration was examined for its effect on glucose-6-phosphatase activity in the liver homogenate of rats. The time course of an increase in this enzyme activity after a single subcutaneous administration of the hormone is shown in Fig. 4. As early as 15 min after hormone administration there was a significant increase in the enzyme activity, which reached a maximum level at 60 min, but began to decrease after that (75 and 90 min).

The effect of CT (80 MRC mU/100 g) on phosphorylase activity in the liver homogenate of rats was examined 1 hr after hormone injection. Control rats received the injection of

Table V. Effect of Calcitonin (CT) on the Liver Glucose-6-Phosphatase and Phosphorylase Activities of Rats

Enzyme		Number of rats	Enzyme activity[a)]
Glucose-6-phosphatase	Control[b)]	6	18.2±0.64[c)]
	CT[d)]	7	25.9±0.34[e)]
Phophorylase	Control	6	28.7±0.90
	CT	7	36.4±0.61[e)]

a) Enzyme activities were expressed as nmol inorganic phosphate liberated per min per mg protein.
b) Control injected sterile distilled water.
c) Each value represents the mean ± S.E.
d) CT (80 MRC mU/100 g) was administered subcutaneously.
e) Differs from respective control mean, $p<0.01$.

distilled water. The phosphorylase activity in liver homogenate was significantly increased by CT administration as shown in Table V.

Discussion

The present studies show that CT caused a significant and dose-dependent increase in the serum glucose of rats. The elevated serum glucose produced by CT is associated with a fall in serum calcium. It is possible that hypocalcemia itself may possess a hyperglycemic effect. EDTA, which has the same effect on the serum calcium as CT, did not cause a significant increase in the serum glucose concentration. Also, prior administration of parathyroid hormone (24 U/100 g) to rats caused a significant decrease in serum glucose concentration which was elevated by CT, while the serum calcium did not change significantly (unpublished results). These results suggest that the effect of CT on the serum glucose does not involve a decrease in the serum calcium.

From the study with glucagon, which induces hyperglycemia in rats, an entirely different pattern is observed in the increase of the serum glucose concentration after CT administration. A single injection of glucagon causes maximum changes in serum elevation in less than 15 min, but begins to decrease after that, while a progressive increase in the serum glucose was kept for 60 min by CT administration. This result indicates that the mechanism responsible for hyperglycemia of CT may exclude glucagon release. In fact, it is known that CT inhibits the secretion of glucagon.[18]

Many of the metabolic parameters involved in the regulation of glucose production by the liver have been investigated in relation to their regulation by glucagon, cyclic AMP, and insulin. Glycogenolysis is promoted by glucagon in the rat liver,[19] while insulin stimulates glycogen formation.[20] Glucagon stimulates gluconeogenesis from lactate or alanine,[21] whereas insulin reduces this effect.[20] On the other hand, it is possible that CT has a capacity to elevate the serum glucose level. The hyperglycemic effect of CT was exhibited in starved rats. The administration of CT is clearly capable of stimulating an increase in phosphorylase activity and a decrease in glycogen level in the liver of rats. Also, CT caused a significant increase in the hepatic glucose-6-phosphatase activity. These fact suggest that CT may promote the production of glucose in the liver.

Presently we do not know the mechanism of CT to stimulate the production of glucose in the liver. Glucagon is known to activate phosphorylase and inactivate glycogen synthetase through its effect on the cyclic AMP concentration. It is reported, however, that CT did not affect the cyclic AMP concentration in the liver cells,[22] but elevated cyclic AMP in the bone[23] and kidney cells,[24] which are target organs of the hormone. Previously we found that CT increased calcium accumulation in the liver cells by inhibiting the efflux of calcium,[25,26] and suggested that the action of CT on liver calcium is not dependent on cyclic AMP.[8] The uptake of calcium by liver cells is related to the regulation of glycogenolysis and gluconeogenesis.[9] Presumably, the exhibition of hyperglycemic effect by CT may be caused by the stimulation of glucose production by the liver through an increase in calcium accumulation in the liver cells induced by CT administration. However, the mechanism of CT to increase the serum glucose remains to be elucidated.

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