

Effects of Insulin and Calcitonin on the Levels of Serum Calcium and Glucose in Rats

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A single intraperitoneal injection of calcium chloride solution (2.0 mg Ca²⁺/100 g body weight) markedly increased both calcium and glucose levels in serum of rats. The increase of both calcium and glucose levels in serum produced by the injection of calcium was significantly inhibited by prior administration of insulin (0.1 U/100 g). In contrary, the level of serum glucose elevated by the injection of calcium was not lowered by the simultaneous administration of insulin and calcitonin (80 MRC mU/100 g), while the serum calcium level was decreased additively. The administration of calcitonin alone to rats caused an increase in serum glucose and a decrease in serum levels. These results suggest that calcitonin has an insulin inhibitory effects not mediated through hypocalcemia in rats.

Keywords—insulin; calcitonin; insulin inhibitory effect of calcitonin; serum glucose; rats;

Introduction

It is well established that calcitonin has a hypocalcemic effect.²⁾ Recently, we found that the serum glucose concentration in rats was significantly increased by the administration of calcitonin.³⁾ But, the mechanism by which calcitonin increases the serum glucose level has not been fully resolved.

On the other hand, it is reported that calcitonin inhibits glucose uptake stimulated by insulin in diaphragm muscle of rats, and that this effect of calcitonin is not mediated by hypocalcemia.^{4,5)} Ziegler, *et al.*⁶⁾ have found that calcitonin provoked a significant impairment of glucose assimilation and insulin output in man. Also, Passeri, *et al.*⁷⁾ reported that the stimulation of insulin secretion by an intravenous glucose load in man inhibited by intravenous infusion of calcitonin. Thus, calcitonin may have inhibitory effect on insulin action.

Therefore, the present studies were undertaken to examine whether the effect of insulin on the serum glucose in rats is affected by the administration of calcitonin. This report describes the interaction of insulin and calcitonin on the increased serum calcium and glucose levels produced by the injection of calcium chloride to rats.

Methods

Male Wistar rats, weighing approximately 100–120 g, were used in this experiment. They were obtained commercially (Nippon Bio. Supp. Center. Co., Ltd., Tokyo). The animals were fed on 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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Insulin (crystalline bovine, 24.3 IU/mg, Sigma Chemical Company), and calcitonin (lyophilized porcine calcitonin, 68 MRC U/mg protein, Armour Pharmaceutical Company) were dissolved in sterile, demineralized water. Insulin solution (0.1 U/ml/100 g body weight) was given as a single intraperitoneal administration to rats, and calcitonin solution (80 MRC mU/ml/100 g body weight) was given subcutaneously.

The animals were bled by cardiac puncture under light ether anesthesia. Blood samples obtained by cardiac puncture were centrifuged immediately after the collection. The serum was separated and analyzed immediately. Determination of calcium was made on 0.1-ml aliquots of serum by atomic absorption spectrophotometer (Perkin-Elmer, Model 303) after precipitation with 10% trichloroacetic acid.⁸⁾ The glucose level in serum was quantitated using Glytel *o*-toluidine reagent.⁹⁾

Results and Discussion

The solution of calcium chloride (2.0 mg Ca²⁺/100 g body weight) was intraperitoneally administered to rats. The animals were killed at varying periods after the injection of calcium. The serum calcium level reached a maximum ($p < 0.01$) 10 min after the injection of calcium, and then began to decrease (Fig. 1). The level was significantly ($p < 0.01$) lower than that of control 45 min after the injection. Meanwhile, the serum glucose level was markedly increased by the injection of calcium (Fig. 1) and this increase still continued even at 60 min after calcium treatment.

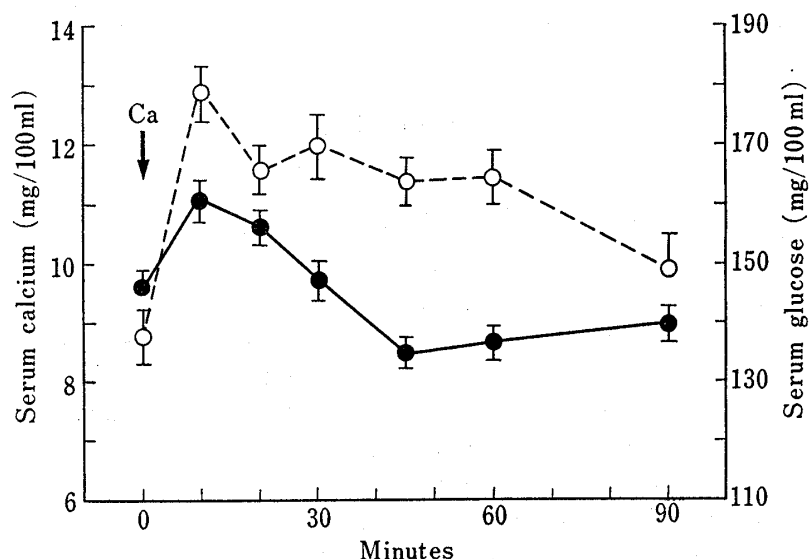


Fig. 1. Effect of the Injection of Calcium Chloride on the Serum Glucose and Calcium Levels in Rats

Calcium chloride (2.0 mg Ca²⁺/100 g) was administered intraperitoneally to rats. The animals were bled at varying period after calcium chloride. Each point represents the mean of 5–6 animals. Vertical lines give the S.E. of means. The levels of glucose and calcium in serum are significantly greater than the control at time periods of 10–20 min ($p < 0.01$). —●—: Serum calcium, ---○---: serum glucose.

Since calcium is required for glucagon secretion,¹⁰⁾ the serum glucose level may be expected to increase after the injection of calcium. On the other hand, an acute increase in serum calcium level caused by the injection of calcium seem to stimulate the secretion of calcitonin from thyroid gland to maintain calcium homeostasis in rats.¹¹⁾ In fact, the serum calcium level at 45 min after the injection of calcium decreased significantly, suggesting that

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there occurred an increase in calcitonin. Recently, we found that the administration of calcitonin significantly increased the serum glucose level.³⁾ It seems that calcitonin sufficiently persists the augmentation of glucose level in serum after injection of calcium to rats, since calcitonin inhibits glucose uptake stimulated by insulin in diaphragm muscle of rats.^{4,5)} Accordingly, prolonged hyperglycemia induced by the injection of calcium may be caused by the elevation of glucagon and calcitonin secretions stimulated by the augmentation of the serum calcium level.

Furthermore, we examined the influence of insulin (0.1 U/100 g) and calcitonin (80 MRC mU/100 g) on the increased serum glucose level caused by the injection of calcium to rats. Insulin or calcitonin was administered 10 min before the injection of calcium. The animals were killed 10 min after the injection of calcium (2.0 mg/100 g). The results (Table I) indicated that prior administration of insulin significantly reduced the increased serum calcium and glucose levels caused by the injection of calcium. However, prior administration of both insulin and calcitonin did not alter significantly the elevated serum glucose level produced by the injection of calcium, while the serum calcium level after the administration of insulin was significantly lowered by the simultaneous administration of calcitonin to rats compared with that of insulin alone. In rats not treated with calcium, the serum glucose level was significantly increased by the simultaneous administration of calcitonin and insulin.

TABLE I. Effects of Insulin and Calcitonin on Serum Glucose and Calcium Levels Increased by the Injection of Calcium Chloride to Rats

Treatment ^{a)}	Serum glucose (mg/100 ml)	Serum calcium (mg/100 ml)
Normal	129.7 ± 4.8 ^{b)}	9.67 ± 0.11
Calcium	174.0 ± 4.4 ^{c)}	11.26 ± 0.17 ^{e)}
Insulin + calcium	108.8 ± 7.0 ^{d)}	10.42 ± 0.16 ^{e)}
Insulin + calcitonin + calcium	182.0 ± 8.5 ^{f)}	9.84 ± 0.23 ^{f)}
Calcitonin + calcium	176.8 ± 4.6	10.11 ± 0.17 ^{d)}
Insulin	121.8 ± 10.2	8.72 ± 0.03 ^{e)}
Insulin + calcitonin	174.7 ± 3.9 ^{g)}	8.19 ± 0.08 ^{g)}
Calcitonin	174.2 ± 13.1 ^{e)}	8.41 ± 0.18 ^{e)}

a) Insulin (0.1 U/100 g) was administered intraperitoneally, and calcitonin (80 MRC mU/100 g) was administered subcutaneously to rats. Calcium chloride (2.0 mg Ca²⁺/100 g) was administered intraperitoneally 10 min after insulin or calcitonin, and the animals were bled 10 min after calcium chloride.

b) Each value represents the mean ± S.E. (obtained from 6 animals).

c) Differs from respective normal mean, $p < 0.01$.

d) Differs from respective calcium mean, $p < 0.01$.

e) Differs from respective calcium mean., $p < 0.01$.

f) Differs from respective insulin + calcium, $p < 0.01$.

g) Differs from respective insulin, $p < 0.01$.

The present results clearly demonstrate that calcitonin prevented the lowering effect of insulin on the serum glucose levels in rats. But calcitonin significantly decreased the serum calcium level after the administration of insulin. These fact suggest that calcitonin has an insulin inhibitory effect not mediated through hypocalcemia in rats.

The mechanism of calcitonin to increase the serum glucose level has not been fully resolved. It is possible, however, that calcitonin inhibits glucose uptake stimulated by insulin since such an effect has been observed in diaphragm muscle of rats.⁴⁾ From the present study, it is conceivable that the hyperglycemia caused by calcitonin administration, at least in part, may be related to the inhibition of insulin action by calcitonin.