

Boc-Pro-Leu-Sar-Pro IV—Boc-Pro (0.35 g, 1.64 mmol) Leu-Sar-Pro-OMe·HCl (0.57 g, 1.64 mmol), NMM (0.20 ml, 1.64 mmol), HOBT (0.32 g, 2.4 mmol) in absolute THF (3.3 ml) were treated with DCC (0.41 g, 2 mmol) in THF (1 ml) and stirred in a similar manner described above and worked up as usual to give an oil which, after chromatography on silicic acid in ethyl acetate-MeOH (90:10), crystallized from ethyl acetate; yield 0.50 g (60%); mp 69–70°; BAW *Rf* 0.47. *Anal.* Calcd. for $C_{25}H_{42}N_4O_7$: C, 58.80; H, 8.29; N, 10.97. Found: C, 58.61; H, 8.09; N, 10.79.

This product was saponified according to the method described above to give an oil which was purified by chromatography on silicic acid in ethyl acetate-MeOH (50:50); yield 0.20 g (40%); mp 100–101°; BAW *Rf* 0.35. *Anal.* Calcd. for $C_{24}H_{40}N_4O_7$: C, 58.04; H, 8.12; N, 11.28. Found: C, 57.84; H, 8.45; N, 11.00.

Pro-Leu-Sar-Pro-HCl V—The above product was then treated with 0.20 ml 4N HCl in dioxan in the conventional manner to give an amorphous product homogeneous on TLC; yield 0.16 g (94%). *Anal.* Calcd. for $C_{19}H_{33}ClN_4O_5$: C, 52.71; H, 7.63; N, 12.94. Found: C, 53.13; H, 8.45; N, 12.02.

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Effect of Insulin on Serum Calcium Concentration in Rats

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A single intraperitoneal administration of insulin to intact and thyroparathyroidectomized rats caused a significant decrease of serum calcium at 10 mU/100 g body weight of the hormone, while it did not show any drop in serum glucose. Administration of 100 mU/100 g of insulin produced a significant decrease of both calcium and glucose in serum. The time course of serum calcium response to insulin is mimiced that of calcitonin with single injections. The present results indicate that insulin has a hypocalcemic effect in rats.

Keywords—insulin; hypocalcemic effect; calcium; intact rats; thyroparathyroidectomized rats

Introduction

Recently, it has been found that calcium provokes a temporary release of insulin in the perfused rat pancreas.²⁾ In contrary, insulin secretion decreases in the absence of extracellular calcium.³⁾ Presumably, calcium may be involved in the regulation of insulin release. Since the acute rise in serum calcium was observed in glucose-stimulated insulin secretion,⁴⁾ it is considered that insulin may cause the changes of serum calcium level.

Therefore, the present studies were undertaken to determine whether the serum calcium level is affected by the administration of insulin. We found that the administration of insulin decreased significantly the serum calcium concentration in intact and thyroparathyroidectomized rats.

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Methods

Animals—Male Wistar rats, weighing approximately 100–130 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 Diets, Co., Ltd., Tokyo) and tap water freely.

Hormones—Insulin (crystalline bovine, 24.3 IU/mg, Sigma Chemical Company), and calcitonin (lyophilized porcine calcitonin, 68 MRC U/mg, Armour Pharmaceutical Company) were dissolved in sterile, demineralized water. The vehicle was injected as control.

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

Analytical Methods—Blood was taken by cardiac puncture and centrifuged immediately. The serum calcium concentration was determined by means of an atomic absorption spectrophotometer (Perkin-Elmer, Model 303) after precipitation with 10% trichloroacetic acid.⁵⁾ The serum glucose level was quantitated using Glytel *o*-toluidine reagent.⁶⁾ The data were subjected to an analysis the variance, and standard error (SE) was calculated from the residual error term. The significance of difference was evaluated using Student's *t*-test.

Results and Discussion

The effect of increasing amounts of insulin on the serum glucose and calcium concentrations is shown in Table I. Insulin (10 and 100 mU/100 g body weight) was intraperitoneally administered to intact rats. They were killed 30 min after the hormone administration. At dose level of 10 mU/100 g, the serum calcium concentration decreased significantly when compared with that of control. At the same dose level, however, the serum glucose level did not change significantly. At dose level of 100 mU/100 g, both the serum calcium and glucose concentrations lowered significantly. Thus, we found that the administration of insulin causes a decrease of calcium concentration in serum of intact rats.

It is well known, however, that the serum calcium concentration is decreased in response to calcitonin.⁷⁾ Therefore, in order to investigate the possibility that the effect of insulin might be due largely to an activation of the release or action of calcitonin, the hormone was intraperitoneally administered to rats which had been surgically thyro-parathyroidectomized 24 hr prior to testing. The animals were bled 30 min after the hormone. At dose levels of 10 and 100 mU/100 g, the serum calcium concentration decreased significantly compared with that of control (Table I). The fact that insulin is still effective after extirpation of the thyroid and parathyroid glands indicates that its hypocalcemic effect is not mediated through the secretion of calcitonin from the thyroid glands. The present findings suggest that insulin

TABLE I. Effect of Insulin on Calcium and Glucose Concentrations in Serum of Intact and Thyro-parathyroidectomized Rats

Treatment	Dose of insulin (mU/100 g)	Serum concentration (mg/100ml)	
		Calcium	Glucose
Intact rats	Control	9.87 ± 0.13 ^{a)}	126.3 ± 0.4
	10	7.84 ± 0.08 ^{b)}	143.4 ± 4.1
	100	8.20 ± 0.14 ^{b)}	108.9 ± 8.8 ^{b)}
Thyro-parathyroidectomized rats	Control	6.13 ± 0.19	154.6 ± 4.4
	10	5.11 ± 0.14 ^{b)}	166.7 ± 6.7
	100	5.04 ± 0.23 ^{b)}	108.0 ± 6.8 ^{b)}

a) Mean ± SEM for 6 animals.

b) Significance from the control, $p < 0.01$ (Student's *t*-test).

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may directly induce the decrease of the serum calcium concentration. However, the mechanism by which insulin decreases serum calcium remains to be elucidated.

It is known that calcitonin has a hypocalcemic effect.⁷⁾ We also compared the time course of insulin and calcitonin actions on the serum calcium concentration (Fig. 1). Insulin (10 mU/100 g) was intraperitoneally administered and calcitonin (80 MRC mU/100 g) subcutaneously to intact rats. Blood was obtained at varying periods after the hormones injection. Figure 1 shows that, as early as 15 min after the administration of insulin or calcitonin, there occurred a significant decrease of the serum calcium concentration. The decrease of serum calcium in response to insulin was maximum at 30 min after the hormone injection, while that of calcitonin was at 60 min.

The present studies clearly demonstrate that insulin, as well as calcitonin, has a hypocalcemic effect in intact and thyroparathyroidectomized rats. Even at dose level (10 mU/100 g) which did not produce the reduction of serum glucose level, insulin caused a significant decrease in the serum calcium concentration. This observation suggests that insulin may also be functioning in a physiological regulation of serum calcium level.

On the other hand, the rise in serum calcium provokes a temporary release of insulin.²⁻⁴⁾ Since the enhancement of serum calcium concentration after the administration of calcium causes an increase in serum glucose level,⁸⁾ it is possible that the hypocalcemic effect of insulin plays an important role in order to prevent the elevation of serum glucose level in rats.

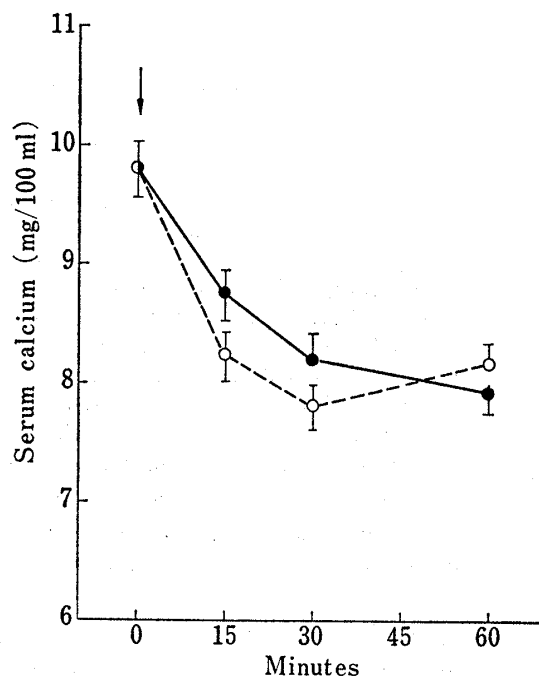


Fig. 1. The Time Course of the Decrease of the Serum Calcium Concentration in Rats treated with Insulin, and Calcitonin

Insulin (10 mU/100 g) was administered intraperitoneally, and calcitonin (80 MRC mU/100 g) subcutaneously. Each point represents the mean of 5-6 animals. Vertical lines give the SEM. The level of serum calcium at 15 min after the administration of all the hormones is significantly lower than the control ($p < 0.01$).

--○--, Insulin; --●--, calcitonin.

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