# Calcitonin Inhibition of Insulin Release from Isolated Rat Pancreatic Islets

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**SUMMARY**

**Calcitonin is known to inhibit secretion of gastrin and insulin in vivo. The objective of this study was to determine whether calcitonin can act directly on pancreatic islets in vitro to inhibit insulin release. Isolated islets were obtained from collagenase-treated rat pancreas, and three peptides (gastrin-releasing peptide, cholecystokinin-8, bombesin) and glucose were used to stimulate insulin release. All agents caused a significant increase in insulin secretion and calcitonin inhibited these responses, but had no consistent effect on basal release. This study provides evidence that calcitonin is an effective inhibitor of insulin secretion and acts directly on islet tissue. DIABETES 1986; 35:58–60.**

The mechanism by which calcitonin induces hypocalcemia is known to involve inhibition of calcium efflux from bone.[1] The mechanisms for inhibition of a variety of gastrointestinal (GI) functions by calcitonin[2–8] are not known. These functions include the secretion of certain GI and pancreatic hormones, e.g., gastrin[1,2,4] and insulin.[3,6–8] In man, calcitonin acts to suppress both basal and stimulated concentrations of insulin.[3,6–8]

The mechanisms by which calcitonin inhibits insulin release in vivo are not known, nor is it known whether calcitonin acts directly or indirectly on the β-cell. The present study was designed to determine whether calcitonin, added directly to functional rat islets in vitro, alters release of insulin from isolated islets.

## MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing approximately 300 g, were purchased from Texas Animal Specialties, Humble, Texas. They were allowed food and water ad libitum and were kept in the laboratory on a regulated light/dark schedule (14 h light/10 h dark; lights on 0500–1900 h). Pancreatic islets were prepared according to the method described by Lacy and Kostianovsky.[9]

In brief, the rat pancreas was removed surgically and subjected to enzymatic digestion for 12–15 min with Hanks' balanced salt solution containing collagenase (Type V, Sigma, St. Louis, Missouri; 25 mg/3 ml). The islets were then washed 3–4 times in 3–5 ml of Hanks' solution before they were harvested. Islets were picked by hand using fine forceps and a dissection microscope. They were preincubated (8 islets/tube) in 1 ml of medium 199 (glucose concentration, 5.5 mM) (Gibco, Grand Island, New York), containing Hanks' salts, for 20 min at 37°C under one atmosphere of air. After preincubation, fresh media (1 ml), containing either gastrin-releasing peptide (GRP) ($10^{-8}$, $10^{-9}$ M), cholecystokinin-8 sulfate (CCK-8; $10^{-9}$ M), bombesin ($10^{-9}$ M), or GRP, CCK-8, or bombesin plus calcitonin ($10^{-8}$, $10^{-9}$ M), were added to the tubes, and the tubes were incubated for 1 h. Media (5.5 mM glucose) alone served as a control. The media were then removed and kept frozen at −20°C until radioimmunoassay of insulin concentration, as previously described.[10]

Synthetic human calcitonin was purchased from BACHEM, Torrance, California, and dissolved (50 μg/ml) in $10^{-3}$ N acetic acid. GRP, CCK-8, and bombesin were also purchased from BACHEM and prepared in distilled water. All incubation tubes in each experiment contained equivalent amounts of acetic acid.

Results are expressed as the mean ± SEM. The Student *t*-test was used to analyze the differences between the means. Differences with a P-value of $<0.05$ were considered significant.

## RESULTS

In comparison to medium alone, GRP stimulated significant release of insulin (Figure 1). Calcitonin ($10^{-8}$ M) inhibited GRP-induced insulin release completely, although calcitonin alone did not alter basal insulin release. At $10^{-9}$ M, calcitonin

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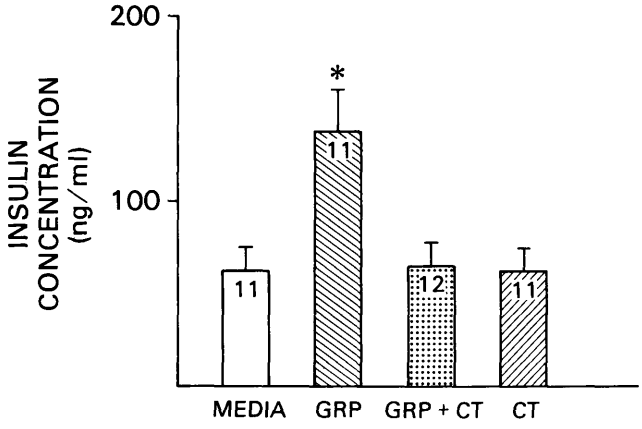


**FIGURE 1. Calcitonin (CT) inhibition of GRP-stimulated insulin release. Both peptides were tested at $10^{-8}$ M. *P < 0.05 versus media, CT + GRP, or CT. In this and subsequent figures, the number within the bar reflects the number of tubes (8 islets/tube) per group. Media contain 5.5 mM glucose.**

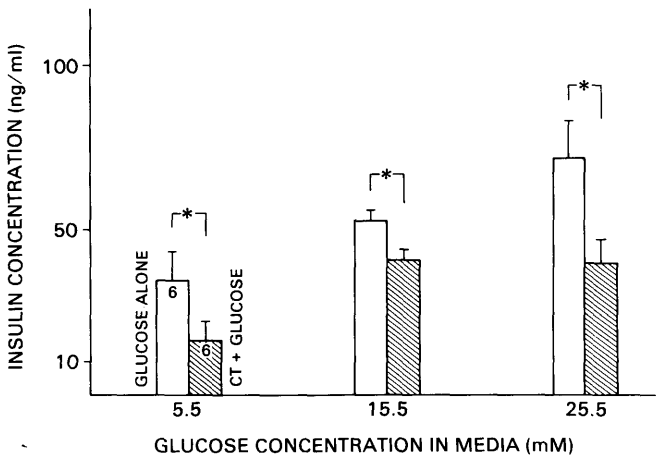


**FIGURE 2. Calcitonin (CT) ($10^{-8}$ M) inhibition of glucose-stimulated insulin release. Open bars, glucose alone; shaded bars, calcitonin plus glucose. *P < 0.05 versus glucose alone.**

also inhibited GRP-induced insulin secretion significantly (with GRP also at $10^{-9}$ M) (Table 1).

Calcitonin also significantly inhibited the insulin response to graded doses of glucose (Figure 2). Although insulin release increased when glucose levels were raised from 15.5 to 25.5 mM, insulin release in the presence of calcitonin seemed to plateau at approximately 40 ng/ml.

Insulin release stimulated by CCK-8 (at $10^{-9}$ M) and bombesin (at $10^{-9}$ M) was also inhibited significantly by calcitonin at $10^{-9}$ M (Figures 3 and 4).

## DISCUSSION

We have previously shown that GRP, CCK-8, and bombesin have potent insulinotropic effects when added to isolated islets.[11] Results from the present study show that calcitonin, at $10^{-8}$ M or $10^{-9}$ M, acts directly on islet tissue in vitro to inhibit the insulin secretion that is evoked by GRP, CCK-8, bombesin, or glucose. In other experiments (data not shown), calcitonin, at concentrations as low as $10^{-11}$ M, inhibited GRP-stimulated insulin release, but did not inhibit basal concentrations of insulin.

The current studies show that calcitonin can act directly on the islet to inhibit insulin secretion, but whether it acts directly on the β-cell is not known. It has been suggested, however, that calcitonin can inhibit gastrin release by stimulating the release of gastric somatostatin.[4] In an earlier study with the isolated perfused rat stomach, eel calcitonin (at $10^{-9}$–$10^{-7}$ M) caused an increase in somatostatin release concurrent with a decrease in gastrin secretion.[4] In the study reported here, we do not know whether the reduction in insulin release, in response to calcitonin, is accompanied by increased release of somatostatin from islet tissue.

It is noteworthy that the circulating levels of calcitonin in the rat vary considerably according to strain, age, sex, and whether the animals have been fasted or have eaten recently.[12-14] In fact, circulating calcitonin levels can be several ng/ml (i.e., on the order of $10^{-9}$ M).[13] Hence, the doses of calcitonin tested in this study are not unreasonably large, especially in light of the fact that many tissues in vitro are less sensitive than their in vivo counterparts. Furthermore, we used human calcitonin rather than the often-employed salmon calcitonin that is more potent compared with mammalian calcitonin in many experimental circumstances.[15] Although rat calcitonin is not available for study, human calcitonin differs from rat calcitonin by only 2 out of 32 amino acid residues.

We have no data to explain the mechanisms by which

TABLE 1
Stimulation of insulin release from isolated rat islets by GRP ($10^{-9}$ M) and its inhibition by calcitonin ($10^{-9}$ M)

| Treatment | N | Insulin (ng/ml) |
|---|---|---|
| Control (media alone) | 12 | 38.7 ± 8.9 |
| GRP ($10^{-9}$ M) | 11 | 71.1 ± 13.3* |
| GRP ($10^{-9}$ M) + calcitonin ($10^{-9}$ M) | 10 | 36.5 ± 8.8 |

Islets were incubated for 1 h as described in MATERIALS AND METHODS. Media contain 5.5 mM glucose.
*P < 0.05 versus GRP or GRP + calcitonin.
N = number of tubes/treatment.

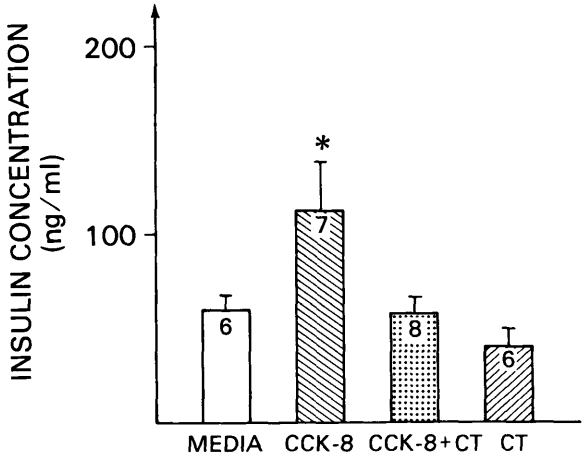


**FIGURE 3. Calcitonin (CT) inhibition of CCK-8-stimulated insulin release. Both peptides were tested at $10^{-9}$ M. *P < 0.05 versus media, CCK-8 + CT, or CT.**

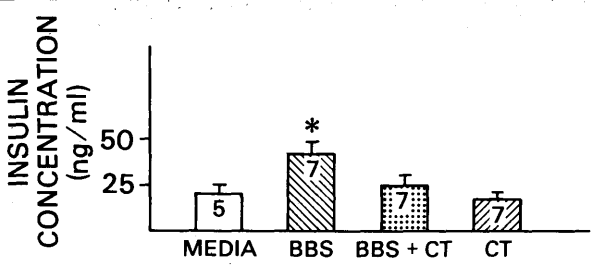


**FIGURE 4. Calcitonin (CT) inhibition of bombesin (BBS)-stimulated insulin release. Both peptides were tested at $10^{-9}$ M. *P < 0.05 versus media, BBS + CT, or CT.**

calcitonin acts to inhibit insulin release. However, the findings do establish calcitonin as an effective inhibitor of insulin secretion, and support its potential role as a physiologic modulator of insulin release.

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