Inhibitory Effects of Synthetic Rat C-Peptide 1 on Insulin Secretion in the Isolated Perfused Rat Pancreas

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TOYOTA, T., ABE, K., KUDO, M., KIMURA, K. and GOTO, Y. Inhibitory Effects of Synthetic Rat C-Peptide 1 on Insulin Secretion in the Isolated Perfused Rat Pancreas. Tohoku J. exp. Med., 1975, 117 (1), 79-83 — The effects of synthetic rat C-peptide 1 on insulin secretion from the isolated perfused rat pancreas was studied. The perfusion technique was according to the procedure described by Grodsky et al. (1963) with a few modifications. After about 20 min of stabilization, the pancreas was perfused for 40 min with synthetic rat C-peptide 1 (100 ng/ml), during which (at 20 min) glucose concentration was switched from 2.7 to 16.6 mM. Insulin response to glucose (16.6 mM) was significantly decreased by the addition of the rat C-peptide 1 in advance when compared with those not pretreated with the C-peptide. In other experiments, the pancreas was exposed to glucose infusion (16.6 mM) for 60 min. 20 min after the start of glucose infusion, the C-peptide (100 ng/ml) was interposed for 20 min. The insulin secretion was reduced by the addition of the C-peptide. It is postulated that the C-peptide may have a role in the regulatory mechanism of the insulin secretion.

It is recognized that proinsulin, some intermediate forms of proinsulin and the C-peptide (Steiner and Oyer 1967; Chance et al. 1968; Rubenstein et al. 1969; Roth et al. 1968; Gutman et al. 1972) are secreted in addition to insulin into the circulatory blood. Insulin and C-peptide were present equimolar in the circulatory plasma (Melani et al. 1970) when evaluated on a molar basis. This suggests that these peptides are secreted in equivalent amounts during emiocytosis of beta secretory granules which may comprise equimolar concentrations of insulin and C-peptide. However, no conclusive physiological significance of circulating C-peptide has been known. Yu and Kitabuchi (1973) reported that C-peptide had no antagonistic or potentiating effect for insulin in fat cells. It is a question whether the presence of C-peptide in the circulation is only a passive consequence of the mechanism of insulin synthesis and secretion, as Melani et al. (1971) stated. The present investigation was undertaken to study the effects of synthetic rat C-peptide 1 on glucose-induced insulin secretion from the isolated perfused rat pancreas.

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MATERIAL AND METHODS

Animals and perfusion system. Male Wistar rats weighing 200–250 g were used in the present study. All animals were fasted for 15–18 hr before the preparation. The pancreas with the proximal portion of duodenum was isolated and perfused according to the procedure described by Grodsky et al. (1963) with some modifications (Toyota et al. 1973). The animals were anesthetized with Nembutal (45 mg/kg body weight). A fixed cannula was inserted into the portal vein, and the pancreas was placed in an incubator. The perfusate was not recycled so that the influence of the gut hormones on the insulin secretion was avoided. The flow rate was maintained constant at 1.8–2.0 ml per min, because an increase in flow rate exceeding two ml per min produced edema of the perfused pancreas. The medium was Krebs-Ringer bicarbonate buffer containing 4.5% dextran and 2.7 mM glucose. Before perfusion, the medium was gassed with a mixture of O₂ and CO₂ (95:5) for 20 min. At intervals indicated in the figures, the perfusate was quickly changed by switching from one circulation medium to another. In all experiments 2 ml samples of perfusate were collected at suitable intervals from the portal vein, and were cooled on ice. After completion of the experiments, the samples were frozen at −20°C until assayed.

Experimental design. 1) After about 20 min of stabilization, the pancreas was perfused for 40 min with rat C-peptide 1 (100 ng/ml), and at 20 min of the perfusion glucose concentration was switched from 2.7 to 16.6 mM (Fig. 1). 2) 20 min after the start of infusion of 16.6 mM glucose the C-peptide (100 ng/ml) was interposed for 20 min (Fig. 2). The results were compared with those of the control experiments without addition of the C-peptide.

Synthetic rat C-peptide 1 used in this study was kindly supplied from Prof. Yanaihara, Shizuoka College of Pharmacy, Shizuoka. Rat insulin was supplied from Novo Research Institute.

Analysis. Insulin was measured by the double antibody procedure of Morgan and Lazarowa (1962), using rat insulin as standard. Statistical evaluations were done according to the Student t-test as well as by linear regression analysis and calculation of coefficients.

RESULTS

Infusion of 2.7 mM glucose brought about no change of insulin secretion. Further addition of the synthetic rat C-peptide 1 to the perfusate did not show any effect on the insulin secretion as seen in Fig. 1. When synthetic rat C-peptide 1 (100 ng/ml) was infused in advance, the insulin secretion stimulated by 16.6 mM glucose was significantly reduced as compared with the control (p<0.01). This showed that the C-peptide suppressed the glucose-induced insulin secretion.

To confirm the inhibitory effects of the C-peptide on insulin secretion, synthetic rat C-peptide 1 (100 ng/ml) was added to the perfusate medium containing 16.6 mM glucose, and this gave rise to a significant inhibition of insulin release (Fig. 2). Also a remarkable off-response was observed when the C-peptide was removed from the perfusate containing 16.6 mM glucose.

DISCUSSION

The present results clearly showed that the synthetic rat C-peptide 1 inhibited the glucose-induced insulin secretion from the isolated perfused rat pancreas, and
Fig. 1. Effects of synthetic rat C-peptide 1 (CP) upon insulin release and effects of glucose (16.6 mM) on the insulin secretion from the perfused rat pancreas in the presence of the C-peptide. The dotted shadow shows the results of the control experiments which are perfused without addition of the C-peptide. When the C-peptide is infused in advance, the insulin secretion stimulated by 16.6 mM glucose is significantly reduced ($p<0.01$, versus the control). Each point represents the mean±S.E. of 6 experiments.

suggest that C-peptide has a possible regulatory role in the insulin secretion. There is evidence that the insulin release may be inhibited when insulin is present in excess (Iversen and Miles 1971; Rappaport et al. 1972). The insulin secretion from the pancreatic $\beta$-cell may be regulated not only by insulin itself but also by C-peptide. It is tempting to assume that this regulation mechanism functions under the physiological condition. Also it may be postulated that, if C-peptide accumulates within the $\beta$-cells due to some defects in its release for a long time, the production and/or release of insulin would be reduced by these excess C-peptide and this condition would subsequently produce a diabetic state.

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Fig. 2. Effects of synthetic C-peptide 1 (CP) upon glucose-induced insulin secretion from the perfused rat pancreas. The dotted shadow shows the control values. When the C-peptide was interposed for 20 min in the presence of 16.6 mM glucose, the glucose-induced insulin secretion is significantly inhibited ($p<0.05$, versus the control). Each point represents the mean±s.e. of 6 experiments.

References

