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Effects of Synthetic Rat C-Peptide in Normal and Diabetic Rats

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Summary. The effects of synthetic rat C-peptide 1 and C-peptide 2 on plasma insulin and blood glucose concentrations in the rat were studied. Infusion of rat C-peptide (500 $\mu g \cdot h^{-1} \cdot kg^{-1}$) diminished glucose induced increase of plasma insulin by 56% (15.2 ± 0.9 versus 6.6 ± 0.6 ng/ml, $p < 0.01$, mean ± SEM). Somatostatin infused at a rate of 50 $\mu g \cdot h^{-1} \cdot kg^{-1}$ body weight inhibited glucose-induced insulin secretion by 33%. In the presence of a mixture of both C-peptides or somatostatin, blood glucose after intravenous glucose was higher than in the control experiments. In alloxan-diabetic rats, C-peptide (160 μg/kg) significantly increased and prolonged the hypoglycaemic effect of exogenous insulin. It is suggested that C-peptide may not be a biologically inert substance.

Key words: C-peptide, insulin secretion, effect of insulin, alloxan-diabetic rats, C-peptide effect in vivo, somatostatin.

Although C-peptide is produced and released from the pancreas in an equimolar concentration to insulin, no definite biological activity has been identified [1]. Several studies failed to show any effect of C-peptide on glucose and lipid metabolism or on insulin secretion [2, 3]. On the other hand, studies in vitro suggest that synthetic rat C-peptide inhibits glucose-induced insulin secretion from rat pancreas [4, 5]. Moreover, Wójcikowski et al. have demonstrated that rat and human C-peptide decrease the release of insulin and glucagon from the isolated perfused rat pancreas [6, 7]. In spite of the inhibition of insulin secretion, C-peptide had no influence on the incorporation of tritiated leucine into proinsulin [8].

The present studies were undertaken to define the action of rat C-peptides on glucose tolerance and insulin release in the rat in vivo.

Materials und Methods

Male Wistar rats, 180–230 g body weight, fed on a normal laboratory diet, were used throughout all experiments.

Normal Rats

The animals were anaesthetized by intraperitoneal injection of pentobarbital (40 mg/kg body weight). The femoral artery and vein were cannulated for blood sampling and infusions of 154 mmol/l NaCl, C-peptide or somatostatin. A mixture (1:1) of rat C-peptide 1 and 2 (500 $\mu g \cdot h^{-1} \cdot kg^{-1}$ body weight) or somatostatin (50 $\mu g \cdot h^{-1} \cdot kg^{-1}$ body weight) in 154 mmol/l NaCl was infused immediately after collection of the first blood sample at 0 time. In control experiments, NaCl was infused at the rate 0.07 ml/min. The infusions were continued throughout the experiments. Glucose (500 mg/kg body weight) was administered at 20 min as an intravenous bolus. Samples of blood (0.3 ml) were taken from the femoral artery for glucose and insulin determination.

Diabetic Rats

Diabetes was induced by a single intravenous injection of alloxan monohydrate (35 mg/kg body weight) dissolved in 154 mmol/l NaCl. After 2 weeks the blood glucose of the animals was elevated > 15 mmol/l glucose. A mixture (1:1) of rat C-peptide 1 and 2 at the dose 80 μg/kg body weight was injected twice into the tail vein. C-peptide was administered 30 min before the injection of insulin and afterwards together with insulin (1 U/kg body weight).

Insulin was determined by a radioimmunological method using dextran-coated charcoal to separate the free antigen [9]. Synthetic rat C-peptides at concentrations ⩽1 μg/ml did not interfere in insulin determination. Rat insulin (Novo, Bagsvaerd, Denmark) was used as a standard. Blood glucose was measured by the ortho-toluidine method.

The results are expressed as mean ± SEM. Statistical differences between means were determined by Student's t-test. The plasma insulin concentrations were transformed logarithmically before analysis by the Student's t-test. Synthetic rat C-peptide 1 (Lot. No. NY-YI-4-34) and rat C-peptide 2 (Lot. No. NY-YI-4-48) were kindly donated by Professor N. Yanaihara, Shizuoka, Japan. The purity of the synthetic rat C-peptides was characterized previously [10].

Results

The infusion of a mixture of rat C-peptide 1 and 2 or somatostatin had no significant effect on fasting plasma

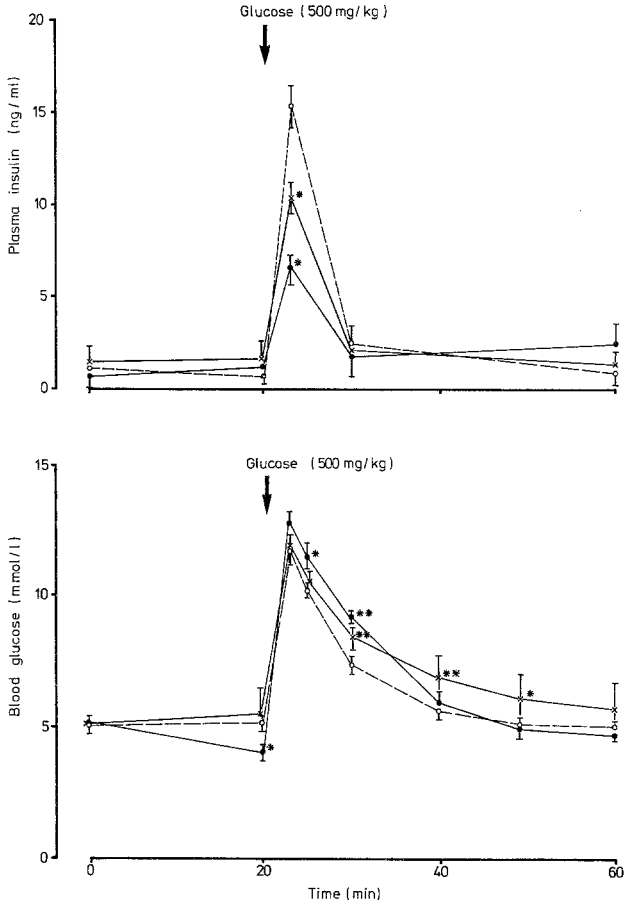

Fig. 1. The plasma insulin and blood glucose responses to intravenous glucose injection during infusion of 154 mmol/l NaCl (O---O), rat C-peptides (●——●) or somatostatin (×——×) into normal rats ($n=6$). p values refer to differences between the value during infusion of 154 mmol/l NaCl and rat C-peptides or somatostatin ($*p<0.05$, $**p<0.01$).

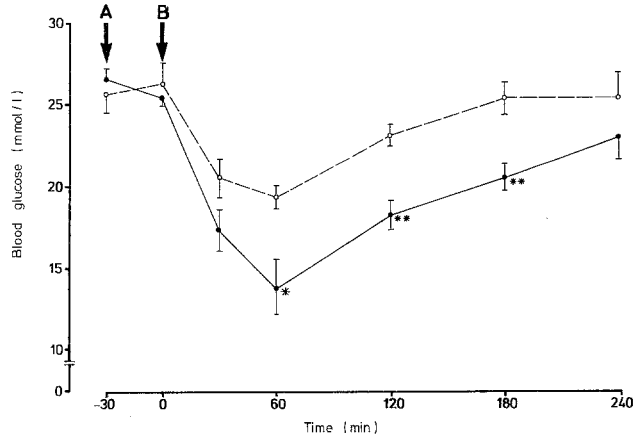

Fig. 2. The effect of rat C-peptides on hypoglycaemic activity of insulin in alloxan-diabetic rats ($n=6$). p values refer to differences between the value following injection of insulin and insulin + C-peptides ($*p<0.02$, $**p<0.01$).
A: intravenous injection of 154 mmol/l NaCl (O---O) or rat C-peptide (●——●); *B:* intravenous injection of insulin (O---O) or insulin + rat C-peptide (●——●)

insulin levels (Fig. 1). However, the increase of plasma insulin induced by intravenous glucose in the presence of C-peptide was reduced by 56% (15.2 ± 0.9 versus 6.6 ± 0.6 ng/ml, $p<0.01$), whereas a reduction of only 36% was observed in the presence of somatostatin. The blood glucose concentrations remained stable in rats receiving an infusion of 154 mmol/l NaCl or somatostatin but in the presence of rat C-peptide it significantly decreased at 20 min from 5.1 ± 0.14 to 4.3 ± 0.22 mmol/l ($p<0.05$). Blood glucose, after intravenous injection of 500 mg/kg body weight of glucose in rats receiving C-peptide, was significantly higher only at 5 and 10 min after the intravenous glucose tolerance test than in animals given glucose alone. The mean blood glucose levels in rats infused simultaneously with somatostatin and glucose were higher at 10 min ($p<0.01$), 20 min ($p<0.01$) and 30 min ($p<0.05$) after glucose administration. Intravenous injection of insulin (1 U/kg body weight) decreased blood glucose in alloxan-diabetic rats from 25.3 ± 1.42 to 19.3 ± 0.71 mmol/l at 60 min (Fig. 2). In animals pretreated with C-peptide, blood glucose following injection of insulin decreased from 24.6 ± 0.59 to 13.7 ± 1.57 mmol/l. The hypoglycaemic effect of insulin alone persisted only 120 min, whereas it was prolonged for 240 min if C-peptide was present additionally.

Discussion

The present finding confirms the previous results in vitro of the inhibitory effect of synthetic rat C-peptide on insulin release [6, 7]. In contrast to our experiments on insulin secretion are the results of Kaneko et al. [11] who found no effect of dog C-peptide on glucose-induced insulin secretion in the dog. This discrepancy may be dependent on the species differences or upon the doses of C-peptide administered. In the dog, C-peptide was infused into the pancreatic artery by adjusting the concentrations to 25 ng/ml in the pancreatic vein in a basal state. In our experiments with rats the effect of C-peptide on insulin release in vivo as well as in vitro was observed at higher concentrations. C-peptide, however, is released by the B cells of the pancreas and for a direct effect on pancreatic islets the local concentration may be more important than the concentration in peripheral blood. As it has been calculated [6], the maximum concentration of C-peptide in the extracellular space of rat islets is extremely high, approximately 10 μmol/l.

In spite of a stronger inhibition of glucose-induced insulin release by rat C-peptide than that effected by somatostatin, the blood glucose concentrations appeared higher than those in control experiments only in the first 10 min. This fact, as well as a decrease of blood glucose concentrations following rat C-peptide administration which was observed also in concentrations corresponding to the physiological range [12], may indicate a hypoglycaemic action of C-peptide itself or an increase of hypoglycaemic activity of insulin through the

C-peptide. Hence, we tested the effect of rat C-peptides administrated in a low dose, on the hypoglycaemic activity of insulin in alloxan-diabetic rats. The lack of a significant effect of C-peptide alone and a greater and prolonged decrease of blood glucose concentrations after insulin injection could be interpreted as an increase in the hypoglycaemic activity of insulin in the presence of synthetic rat C-peptides. Although the action mechanism may be different, it cannot be excluded that the insulin binding to receptors becomes increased or that insulin degradation is decreased.

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