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Pancreastatin (33-49) enhances the priming effect of glucose in the rat pancreas

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Abstract. Short-term exposure to glucose increases insulin secretion during subsequent stimulation. We investigated the effect of the new regulatory peptide pancreastatin on this priming effect of glucose in the perfused rat pancreas. Pancreastatin (33-49) at a concentration of 10^{-8} M inhibited insulin release when stimulated by glucose at a concentration of 16.7 mM. However, after a second pulse of 16.7 mM glucose, pancreastatin potentiated the priming effect of glucose on insulin secretion. The modulation of insulin secretion by pancreastatin results in a potentiation of the priming effect of glucose in the rat pancreas, suggesting a role for pancreastatin in the adaptation of the B cell to glucose-stimulated insulin secretion.
Key words. Pancreastatin; perfused rat pancreas; glucose priming; insulin secretion.

Pancreastatin, a 49 amino acid peptide isolated from porcine pancreas[1], has been shown to inhibit insulin release both in vivo and in vitro[1-3]. In addition, extrapancreatic effects have been described, such as inhibition of parathormone release[4] and stimulation of hepatic glycogenolysis in vivo[5,6] and in vitro[7]. Its mechanism of action is still not completely understood. However, studies using insulin-secreting RIN m5F cells have shown that a pertussis toxin-sensitive G protein[8] as well as mobilization of Ca^{2+} [9] may play a role in the inhibition of insulin secretion by pancreastatin.

It has been reported that prior exposure to glucose has priming or 'memory' effect in the B cell[10]. Thus, a short period of glucose stimulation may potentiate the secretory response of the B cell to a second pulse of glucose, although the mechanisms involved in this feedback action of glucose on insulin secretion are poorly understood.

Pancreastatin seems to inhibit the first phase of insulin secretion in the perfused rat pancreas[1] without modifying the second phase. To investigate the influence of pancreastatin on the priming effect of glucose in the first phase of insulin secretion, we studied the dynamics of the secretory response to two 5-min pulses of 16.7 mM glucose, separated by 15 min of perfusion with 2.7 mM glucose.

Materials and methods

Bovine serum albumin (BSA, fraction V) and dextran (T-70) were from Sigma (St Louis, Missouri). Porcine pancreastatin (33-49) was from Peninsula Laboratories Europe, Ltd (Merseyside, U.K.). Male Wistar rats (250–300 g) were used. Animals were fed a standard diet ad libitum. Before the experiments the rats were fasted for 16 h overnight. The pancreas was isolated from each anaesthetised rat (50 mg/kg) and perfused by the procedure of Sussman et al.[11] with some modifications[12]. The isolated pancreases were perfused at a rate of 2.5 ml/min with Krebs-Ringer bicarbonate buffer, pH 7.4, containing 1% bovine serum albumin (BSA), 2% dextran and glucose as specified under 'Results'. The buffer solution was pumped through the aorta at a perfusion pressure of 20 mmHg, and the total portal effluent was collected every 2 min, chilled in ice and stored at −20 °C. Pancreastatin (33-49) (10^{-8} M) was dissolved in the perfusion medium before infusion. Glucose was infused by a precision pump via a side arm so that the glucose concentration of the medium could be changed rapidly. Insulin secretion was measured as previously described[12]. The results are presented as mean ± SEM. Analysis of variance (ANOVA) and the Bonferroni post-test were used to test the degree of significance of differences between groups.

Results and discussion

We have used a brief period of glucose exposure (5 min) to study the first phase of insulin secretion. We have employed the 33-49 C-terminal synthetic fragment of porcine pancreastatin because of its high degree of homology with the putative amino acid sequence of rat pancreastatin and its known ability to inhibit the first phase of insulin secretion in the perfused rat pancreas[1]. The figure shows the dynamics of the insulin secretory response to 2.7 mM and 16.7 mM glucose in the absence and presence of 10^{-8} M pancreastatin. A 5-min pulse of 16.7 mM glucose significantly ($p < 0.01$) enhanced the insulin response to a second pulse of 16.7 mM glucose. When pancreastatin was added to the perfusion medium, the insulin response to 16.7 mM glucose was significantly ($p < 0.005$) inhibited. However, pancreastatin significantly increased ($p < 0.05$) the

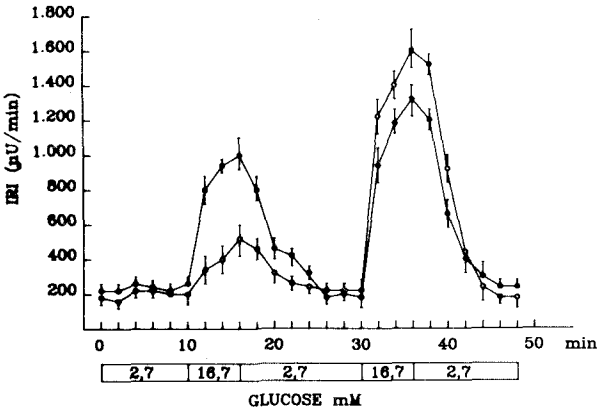

Figure. Secretion of insulin from isolated rat pancreas in response to two pulses of 16.7 mM glucose. Effect of 10^{-8} M pancreastatin (open circles) on the priming action of glucose (closed circles). Mean ± SEM of 6 pancreases perfusions are shown.

enhanced insulin response to a second pulse of 16.7 mM glucose. These results agree with the time-dependent potentiation or memory of the B cell response by glucose as described in literature[10, 13, 14], and suggests a role for pancreastatin in the adaptation of the B cell to glucose stimulation of insulin secretion.

As expected[1], pancreastatin did not modify the rate of insulin secretion when the glucose concentration of the perfusion medium was 2.7 mM, showing a lack of effect on non-stimulated insulin release. On the other hand, pancreastatin inhibited the first phase of insulin secretion as previously described[1]. However, this inhibitory effect turned out to be transitory, and after the second glucose challenge, pancreastatin behaved as a potentiator of the priming effect of glucose on insulin secretion. The mechanisms involved in the action of pancreastatin are not completely understood, but since the priming effect of glucose is supposed to be due to a realignment of secretory granules to positions more favorable for exocytosis[10], pancreastatin does not seem to inhibit insulin release by interfering with this action. Rather, it is possible that pancreastatin acts by hindering exocytosis at a later stage, such as the fusion and lysis of the granule, events that have been reported to be regulated by calmodulin and cyclic AMP respectively[15]. The fact that pancreastatin can mobilize intracellular calcium in insulin-secreting cells in a fashion similar to α_2-adrenergic agonists[9] and epinephrine[16] further supports this hypothesis of a pancreastatin action close to the exocytotic event, as has been proposed for epinephrine[17].

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