Potentialiation by insulin of the acetylcholine-induced secretory response of the perfused rat pancreas

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ABSTRACT

The influence of exogenous insulin on acetylcholine (ACh)-induced secretory responses was studied using the isolated perfused rat pancreas preparation. Addition of bovine insulin (50 mU/ml) to the perfusing solution potentiated the action of ACh (3×10^{-8} M) to increase both pancreatic juice flow and release of the digestive enzymes, i. e. amylase, trypsin and chymotrypsin.

KEY WORDS insulin / acetylcholine / pancreatic acinar cell

It is well established that the release of digestive enzymes from the pancreas is physiologically controlled both by cholecystokinin-pancreozymin (CCK-PZ) and by vagal activity. There is evidence that exogenous insulin potentiates the action of CCK-PZ on the pancreatic acinar cell in the isolated perfused rat pancreas (3, 5, 6, 10), as well as in the anesthetized rat (3, 5). Furthermore, our recent study using the perfused pancreas demonstrated that raising the glucose concentration in the perfusing solution from 2.5 to 17.5 mM increased endogenous insulin release and potentiated the CCK-PZ-induced exocrine secretory response (10). Evidence was presented that this effect of glucose on the exocrine pancreas was mediated by endogenous insulin release (10). In the anesthetized pig, we showed that gradual elevation of the glucose level of circulating blood increased plasma insulin and potentiated the secretion of pancreatic enzymes in response to vagal stimulation (7). All of the previous studies used CCK-PZ as the secretagogue. We now demonstrate that insulin also potentiates the ACh-induced secretory responses of the isolated

perfused rat pancreas.

The isolated and perfused pancreas of rat was prepared as reported previously (4). The composition of the standard Krebs-Henseleit solution was shown in the preceding paper with the perfusing flow rate held constant at 2 ml/min (8). Bovine serum albumin (Sigma Co., St. Louis, Mo) was added to the perfusate at a concentration of 0.25 %. ACh was dissolved directly in the perfusing solution. Estimation of flow of pancreatic juice, total protein output and amylase output were the same as in the preceding paper (8). Trypsin and chymotrypsin were assayed using α -N-benzoyl-DL-arginine-p-nitroanilide and N-glutaryl-L-phenylalanine-p-nitroanilide as substrates (1, 2), with the aid of the Bratton-Marshall reaction to increase sensitivity (9). Trypsin was activated with partially purified enterokinase (Miles Laboratories) added at a concentration of 0.5 mg/ml for 45 min and chymotrypsin with trypsin (Sigma Co., Type XI-chymotrypsin inactivated) at a concentration of 30 μ g/ml for 30 min. In both cases activation was carried out at 37°C in plastic tubes at a constant protein concentration followed by addition of the substrate and incubation for 15 or 30 min for trypsin and chymotrypsin, respectively. Results were expressed in units defined as μ mol of the p-nitroaniline product per min at 37°C.

The isolated pancreas was first perfused for

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Fig. 1 Acetylcholine-induced secretion of pancreatic juice, protein, amylase, trypsinogen (shown by trypsin activity) and chymotrypsinogen (shown by chymotrypsin activity) from pancreata perfused with the standard solution (left column) or with the solution containing 50 mU/ml bovine insulin (right column). The horizontal solid line and the open horizontal bars indicate the periods insulin and 3×10^{-8} M acetylcholine were present respectively. Each value represents the mean $\pm$ S.E. of a 10 min measurement from 5 experiments.

40 min with the standard Krebs-Henseleit solution to allow the secretory response to reach steady values. Pancreatic juice was collected from the common duct at 10 min intervals, its volume measured and the content of protein and enzymes determined. The vascular perfusion was then switched to the same solution containing 3×10^{-8} M ACh and the perfusion was continued for an additional 50 min. As shown in Fig. 1, ACh caused a rapid increase in all secretory parameters. The influence of exogenous bovine insulin was examined by adding insulin (50 mU/ml) 20 min before the initiation of continuous stimulation with ACh.

Juice flow and the output of total protein, amylase, trypsin and chymotrypsin induced by 3×10^{-8} M ACh in the presence of insulin were about twice as much as the corresponding ACh-induced secretory responses in the absence of insulin (Fig. 1B). Analysis of the corresponding secretory responses using Student's *t* test showed that the potentiating influence of insulin on the ACh-induced secretory responses was significant ($P < 0.05$).

In the present study we have shown that exogenous bovine insulin (50 mU/ml) potentiates the secretory response of the exocrine pancreas induced by 3×10^{-8} M ACh. This is compa-

able with our previous findings that insulin potentiated the CCK-PZ-induced secretory responses. Two new and important observations have thus been made; first, that insulin potentiates the action of two different types of secretagogues (ACh and CCK-PZ), and second that insulin potentiates in a parallel manner the release of three different digestive enzymes. While previous studies were compatible with a possible locus of insulin action at the CCK-PZ receptor, this now seems unlikely. Recently, the pancreatic CCK-PZ receptor has been characterized and insulin was shown not to alter the binding of radioiodinated CCK-PZ (11). Now we report that insulin alters the secretory response initiated by ACh which is known to activated secretion via a muscarinic cholinergic receptor.

Since insulin is known to regulate the synthesis and content of pancreatic amylase (12), previous secretory studies left open the question of whether the effect of insulin to potentiate amylase was unique to that enzyme. The present study now shows a parallel increase in trypsin and chymotrypsin secretion. It is generally held that the actions of both CCK-PZ and ACh are mediated by intracellular Ca^{2+} (13) and that all pancreatic digestive enzymes are released in a parallel manner. The present study suggests that insulin may act on a process common to both secretagogues either to facilitate the stimulus-induced increase in intracellular Ca^{2+} or to facilitate the action of Ca^{2+} to induce exocytosis.

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