INSULIN SECRETION IN DOGS WITH EXPERIMENTAL PANCREATITIS IN RESPONSE TO INTRAPANCREATIC INJECTION OF GLUCOSE

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Disturbance of the internal secretory function of the pancreas in chronic pancreatitis has attracted the attention of many investigators. Most of them have judged the functional state of the insular apparatus from the blood sugar curves after glucose loading. Only in the last few years have investigations based on the radioimmune determination of insulin been published [1-8, 10, 11]. However, data in the literature on changes in carbohydrate tolerance and the plasma insulin concentration in chronic pancreatitis are heterogeneous and often contradictory. Among the possible explanations of this fact are evidently differences in the techniques used as an approach to the study of the endocrine function of the pancreas and the inadequacy of data on insulin secretion based on the study of the blood insulin concentration in peripheral vessels.

The writers' previous investigation [1] showed that the dynamics of insulin secretion is best reflected by the insulin concentration in blood from the efferent vessel of the pancreas. After intravenous injection of glucose into dogs with chronic pancreatitis a definite tendency was found for the level of insulin secretion to fall despite no change in glucose tolerance. The first phase – the phase of rapid insulin release – was particularly sharply inhibited [2].

The object of this investigation was to study the early changes in the insulin concentration in the pancreatic vein of dogs with experimental pancreatitis after rapid injection of glucose directly into the pancreatic artery.

EXPERIMENTAL METHOD

Experiments were carried out on 14 anesthetized dogs weighing 18-20 kg after overnight food deprivation. The operative procedure was the same as that used previously [9]. After laparotomy a silicone-treated catheter was introduced into the main branch of the superior pancreaticoduodenal vein against the blood flow (Fig. 1), and its distal end was connected to a catheter introduced into the femoral vein (to ensure a closed system of circulation of blood). During taking of blood samples from the pancreaticoduodenal vein, the catheters were disconnected. To prevent the blood from clotting in the catheter, another catheter was introduced into the duodenal branch of the superior pancreaticoduodenal vein. Throughout the experiment, heparinized saline was introduced from it through a drip system. A thin Teflon catheter was then passed into the superior pancreaticoduodenal artery through the gastroepiploic branch. This catheter was connected to an infusion pump, by means of which the heparinized salt solution was injected at the rate of 0.25 ml/min. Perfusion with heparinized saline was stopped 1-1.5 h after the end of the operation, before the beginning of taking of the blood samples, and blood flowed freely for 15-20 sec from the pancreatic vein. Several initial blood samples were then taken into heparinized tubes, after which glucose was injected rapidly into the pancreatic artery in a dose of 2 mg/kg body weight in 0.2 ml of physiological saline. Next, changing the tubes every 10 sec, blood samples were collected for 90 sec. Subsequent blood samples were collected 2, 3, 5, 10, and 20 min later. The experiment was repeated 3 or 4 times at intervals of 30 min (by which time the glucose and insulin levels had returned to their initial values). The blood samples thus obtained were centrifuged for 10 min at 4°C and then frozen at –20°C. Glucose was determined by the o-toluidine method, insulin by a radioimmune method using standard kits (from CEA-IRE-Sorin, France). Experimental pancreatitis was induced by injection of

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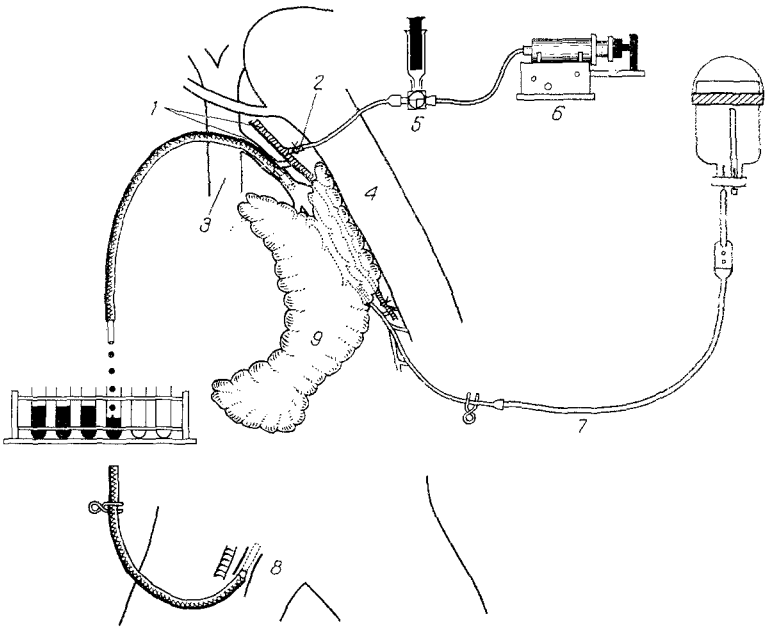

Fig. 1. Perfusion of the pancreas in situ. 1) Superior pancreaticoduodenal artery and vein; 2) gastroepiploic artery; 3) portal vein; 4) duodenum; 5) three-way cock; 6) infusion pump; 7) heparinized physiological saline; 8) femoral vein; 9) pancreas.

autologous bile (0.1 ml/kg) into the pancreatic duct, which was briefly ligated. The operation wound was then sutured in layers under sterile conditions. The experiments with perfusion of the pancreas in situ in these animals were carried out 1 month later. The presence of pancreatitis was confirmed by morphological investigation of the gland.

EXPERIMENTAL RESULTS

After injection of glucose into the control animals a rapid rise in its level in blood from the pancreatic vein was observed, starting from the 5th second, and it reached a maximum at the 10th second (Fig. 2). By this time the plasma insulin concentration began to show a definite increase. The maximal insulin response was observed 40-50 sec after intraarterial injection of glucose, when its concentration was beginning to fall. The insulin level in the pancreatic vein returned to its initial value with effect from the 90th second. The rate of the blood flow remained significantly unchanged throughout the experiment. In experimental pancreatitis the character of the early changes in the glucose and insulin concentrations in blood from the pancreatic vein in response to rapid injection of glucose differed from that in the control. The maximal rise in the glucose level took place, just as in the control animals, at the 10th second. However, the glucose concentration, both during fasting and during the 90 sec after intraarterial injection of glucose, was lower in these animals than in the control. The results of these investigations correspond to previous observations showing some increase in tolerance to glucose in the intravenous glucose tolerance test on dogs with experimental pancreatitis. Insulin secretion during rapid injection of glucose into the pancreatic artery was inhibited. The rise in the insulin concentration was slower, the curve was flatter, and the maximum was delayed and was less well defined. By the 90th minute there was no tendency for restoration of the original insulin level. The rate of the blood flow in experimental pancreatitis was indistinguishable from the control throughout the experiment. The reserve insulin production is known to be reflected in the ratio of its concentration to the glucose concentration. During intraarterial injection of glucose, this index fell distinctly in the dogs with experimental pancreatitis. The rise in the glucose level at the time of the maximal increase in its concentration (at the 10th second) compared with its initial level was less marked in the dogs with experimental pancreatitis (274 ± 45.5 mg %) than in the control animals (353 ± 28.6 mg %). The ratio of the glucose level at the time of maximal rise of its concentration to the initial level showed no significant change under these circumstances (4.73 ± 0.48 in the control, 5.0 ± 1.06 in dogs with pancreatitis). The rise in the insulin level at the time of the maximal increase in its concentration was sharply reduced (control 1793 ± 637 microunits/ml). The ratio of the insulin level at the time of its maximal value to the initial value in dogs with experimental pancreatitis was reduced by more than half (8.97 ± 2.58 in the control, 3.9 ± 0.97 in dogs with pancreatitis). The total increase in insulin

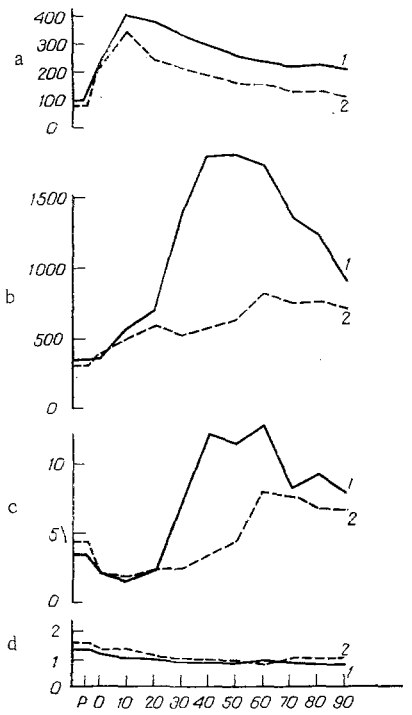

Fig. 2. Dynamics of insulin and glucose concentrations, their ratio, and velocity of blood flow in pancreaticoduodenal vein after rapid injection of glucose into pancreatic artery of dogs with experimental pancreatitis. Abscissa: time of taking blood samples (in sec); ordinate: a) glucose concentration (in mg %); b) insulin concentration (in microunits/ml); c) ratio of insulin concentration (in microunits/ml) to glucose concentration (in mg %); d) velocity of blood flow (in ml/10 sec). 1) Control; 2) pancreatitis.

and glucose is an important indicator of the internal secretory function of the pancreas. In dogs with experimental pancreatitis the total increase in insulin over a period of 90 sec was sharply reduced (7126 ± 3037 microunits/ml in the control, 3268 ± 1013 microunits/ml in dogs with pancreatitis). The total increase in the glucose in the experimental animals, however, was not increased but, on the contrary, was actually slightly reduced (1720 ± 335 mg % in the control, 1082 ± 2.81 mg % in dogs with pancreatitis). The insulinogenic index (the ratio of the total increase in the insulin to the total increase in glucose) over this period was considerably reduced in the dogs with experimental pancreatitis (3.27 ± 1.09, compared with 6.98 ± 3.49 in the control), in agreement with the view expressed previously that there is a definite deficiency in the insulin-secreting mechanism in experimental pancreatitis.

The results of the present investigation are evidence of a disturbance of the early insulin response to glucose in animals with experimental pancreatitis. They confirm previous observations [2] obtained by the intravenous glucose tolerance test, according to which the level of insulin secretion is reduced in experimental pancreatitis, especially in the first phase of the acute release of insulin. It must be assumed that the rapid outflow of insulin was disturbed in experimental pancreatitis, probably as a result of its mobilization before a meal.

The results also indicate that the potential prospects for development of diabetes in animals with experimental pancreatitis can be more clearly revealed by the study of the dynamics of insulin secretion after injection of glucose directly into the pancreatic artery. This experimental model of perfusion of the pancreas in situ is also promising for the study of the action of various substances which stimulate insulin secretion.

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EFFECT OF 5-FLUOROURACIL ON ACTIVITY OF SOME PANCREATIC AND SERUM ENZYMES IN RATS WITH ACUTE PANCREATITIS

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A considerable increase in the incidence of acute pancreatitis (AP) has been recorded everywhere in recent years, and the mortality in this disease still remains high [4, 5]. Methods of conservative and operative treatment of AP have not been finally settled. In the pathogenesis of AP a basic role is played by activation of pancreatic enzymes, followed by injury to the organ by activated proteinases [1, 2].

The main structural feature distinguishing the exocrine cell of the pancreas is the well-marked development of its endoplasmic reticulum and the high density of its ribosome populations [10], essential for enzyme production and secretion. This is responsible for the high rate of protein synthesis in the acinar cells of the pancreas, directly proportional to the rate of RNA synthesis [13].

It was accordingly decided to study the effect of 5-fluorouracil (5-FU) on the activity of some pancreatic enzymes in rats with AP. The molecular mechanism of the action of 5-FU is based on the fact that, first, it inhibits DNA synthesis by depressing activity of thymidylate synthetase, an enzyme which methylates deoxyuridylic acid into deoxythymidylic acid, and second, that it blocks protein synthesis by incorporating a metabolite of uracil instead of normal uracil into the newly synthesized RNA.

It has been shown [9] that treatment of animals with acute experimental pancreatitis by 5-FU inhibits enzyme secretion by the pancreatic acinar cells and that in small doses 5-FU prevents digestion of pancreatic tissue by activated pancreatic proteinases. The authors cited [9] tested the effect of 5-FU on the course of AP only as reflected in the change in the serum α-amylase level and they did not measure the activity of other secretory, as well as nonsecretory, intracellular pancreatic enzymes.

To obtain a deeper insight into the mechanism of action of 5-FU on the course of AP, its effect on the activity of several serum and pancreatic enzymes was studied, including an enzyme specific for the pancreas, namely transaminase (TA).

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