PLASMA INSULIN CONCENTRATION IN THE FEMORAL, HEPATIC, AND PANCREATICO-DUODENAL VEINS IN DOGS DURING STANDARD AND CORTISONE GLUCOSE TOLERANCE TESTS

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Comparison of glucose tolerance tests carried out with or without cortisone showed the advantage of the former when testing the internal secretory function of the pancreas. With the aid of this test the insulin secretion during glucose loading was found to be biphasic. The dynamics of the blood insulin level in the pancreatico-duodenal vein reflects insulin secretion by the pancreas better than the dynamics of its concentration in the blood of the peripheral veins.

KEY WORDS: insulin secretion; cortisone glucose tolerance test.

This paper describes the results of a comparative study of the dynamics of the plasma insulin concentration in blood taken from the superior pancreatico-duodenal, hepatic, and femoral veins during glucose tolerance tests (GTT). This method gives the most adequate idea of the internal secretory function of the pancreas. Its advantage over the assessment of insulin secretion *in vitro* is that the natural circulation of the blood is preserved, which means that the observations can be continued for much longer.

EXPERIMENTAL METHOD

For 2 weeks dogs (18-30 kg) received a standard diet, but they were starved for 24 h before the experiment. Under hexobarbital anesthesia (0.5 mg/kg, intramuscularly) after morphine premedication (1 ml/kg), the vessels were catheterized and for 90 min 100 ml physiological saline was injected by subcutaneous drip. The first blood samples were then taken from the catheters and glucose solution was injected for 10 min into the femoral vein of the contralateral limb in a dose of 1 g/kg body weight. In the experiments with the cortisone GTT (CGTT), 2 h before the glucose was injected, 2.5 mg/kg cortisone was injected through a gastric tube. Blood samples were taken immediately after the end of the glucose infusion and at definite time intervals during the next 2.5 h. To calculate the insulin production the time taken for the blood to flow from the superior pancreatico-duodenal vein was recorded. The blood was centrifuged for 1 h at 4°C. The plasma was frozen at −20°C and used for determination of immunoreactive insulin (IRI) by means of the standard Insulin Immunoassay Kit. Parallel determinations were made of the blood sugar by the orthotoluidine method.

EXPERIMENTAL RESULTS AND DISCUSSION

The highest fasting blood sugar was observed in the hepatic vein. In the pancreatic and femoral veins its concentration was about equal. After injection of glucose the blood sugar rose sharply in all the veins, but most of all in the blood of the pancreatic vein and least in that of the femoral vein.

Injection of cortisone increased the blood sugar concentrations in all the veins tested to some extent. Subsequent injection of glucose was followed by a greater increase in the

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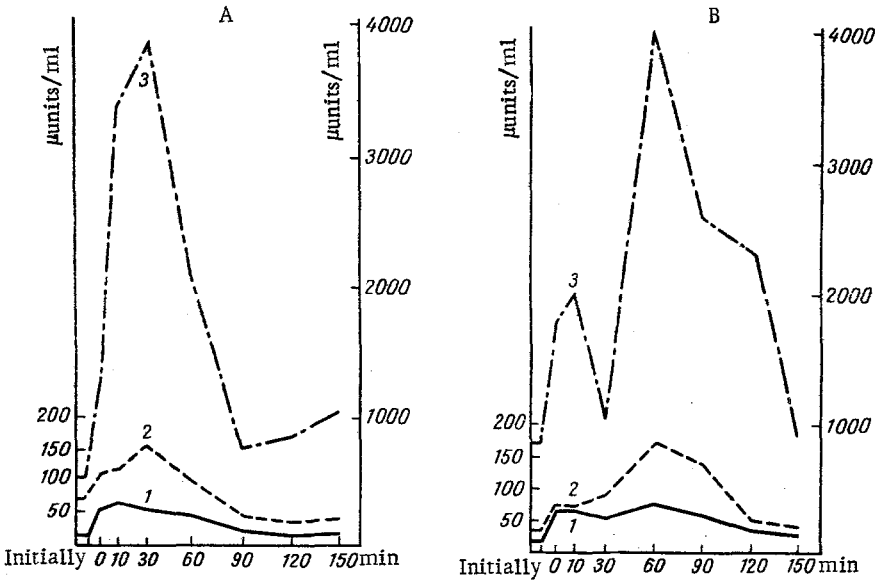

Fig. 1. Dynamics of plasma IRI concentration in blood from superior pancreatico-duodenal, hepatic, and femoral veins of dogs during standard (A) and cortisone (B) glucose tolerance tests. 1) Hepatic vein; 2) femoral vein; 3) pancreatic-duodenal vein. Mean results of tests on six dogs. Ordinate: left, IRI concentration in blood plasma from femoral and hepatic veins; right, from pancreatico-duodenal vein (in μunits/ml); abscissa, time of taking blood samples after end of glucose infusion (in min).

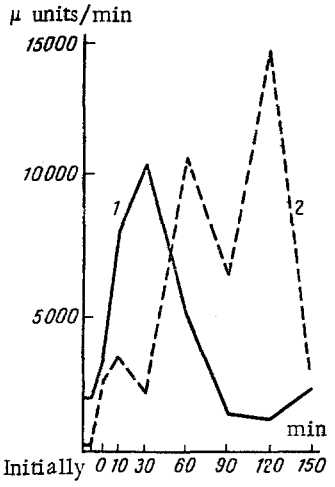

Fig. 2

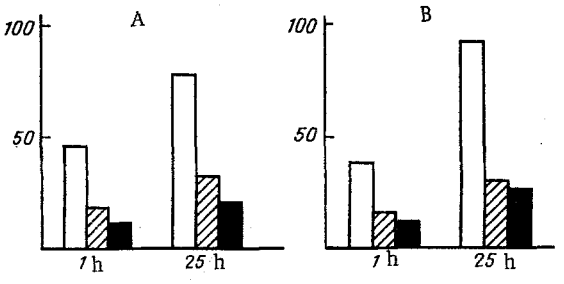

Fig. 3

Fig. 2. Insulin production by pancreas of dogs during standard (1) and cortisone (2) glucose tolerance tests. Ordinate, insulin production (in μunits/min); abscissa, the same as in Fig.1.

Fig. 3. Ratio between total increase in insulin and that of glucose in pancreatico-duodenal (unshaded columns), femoral (shaded columns), and hepatic (black columns) veins in dogs during standard (A) and cortisone (B) glucose tolerance tests (mean results of six experiments).

blood sugar than when glucose was given without cortisone. At the end of the CGTT the blood sugar fell below its initial level.

The highest fasting insulin concentration (mean 527 μunits/ml) was found in the blood of the pancreatic vein. After the blood had passed through the liver, where intensive destruction of the hormone takes place, the insulin concentration in the plasma fell sharply. On

average it was higher in the blood from the femoral vein than in blood from the hepatic vein (75 and 19 μunits/ml, respectively). Intravenous injection of glucose led to an increase in IRI in the blood plasma in all the veins studied (Fig. 1).

Changes in the IRI concentration during the standard GTT were slightly biphasic in character. The first peak in the IRI concentration in blood from the hepatic vein was observed at the 10th minute, and in the other vessels at the 30th minute of the GTT and it was evidently connected with the acute liberation of the hormone from the reserves. A second peak of insulin concentration was found in blood of the pancreatic vein after 120-150 min, but it was much smaller than the first. The second peak of IRI was absent in blood from the hepatic vein.

A second peak of insulin concentration in the pancreatic vein after glucose infusion was described previously [3, 6]. The blood insulin level in the peripheral vessels is known to depend not only on its secretion by the pancreas, but also on the intensity of the binding of insulin with and its liberation from the plasma proteins [1] and of its metabolism in the tissues [5]. After injection of cortisone the plasma IRI level in the pancreatic vein was raised considerably even before the glucose infusion began, it was unchanged in the blood from the hepatic vein, and lowered in blood from the femoral vein. The ratio between the insulin concentrations in blood from the three veins did not change significantly during the CGTT. After intravenous injection of glucose after administration of cortisone the IRI concentration again was increased the most in blood from the pancreatic vein.

By contrast with the standard GTT, changes in the insulin concentration in all the vessels during the CGTT were of a well-marked biphasic character, as was most clearly revealed by analysis of blood from the pancreatic vein. The time of appearance of the maxima of the blood IRI concentration in the pancreatic and femoral veins during the CGTT was shifted nearer to the time of glucose injection. The second peak of insulin concentration was much larger than the first and was recorded sooner during the CGTT than during the standard GTT. By the 150th minute the insulin concentration has usually started to fall to its initial values.

Preliminary administration of cortisone thus led to an earlier and more clearly defined biphasic character of changes in the insulin concentration in all the veins.

An important characteristic of insulin secretion is the ratio between the IRI and glucose concentrations. At the beginning of the standard GTT this ratio was lowered, for the glucose concentration was sharply increased at that moment. Later (from 30 to 60 min) there was an increase in the insulin concentration and a decrease in the glucose level, so that the IRI/glucose ratio rose considerably; however, after 90 min it fell again in the blood from all three veins. A second increase of this index was observed after 120-150 min in the pancreatic and femoral veins, corresponding to the second peak of insulin secretion. During the CGTT the duration of the second increase in the IRI/glucose ratio rose to 120 min, after which it began to fall. This index also reached a higher maximum in the CGTT than in the standard GTT.

Insulin production, calculated with allowance for the insulin concentration and the velocity of the blood flow in the pancreatic veins [4] was much higher in the CGTT than in the standard GTT and was multiphasic in character (Fig. 2).

Another indicator of pancreatic secretory function is the ratio between the total increases in the plasma insulin and glucose during the glucose tolerance tests [2]. The total increase of insulin in the course of both tests was greatest in the pancreatic vein. The increase in glucose in all veins was less marked, but in the CGTT it was more marked. The ratio between the increase in IRI and the increase in glucose in the three veins was greater after 2.5 h during the two tests than after 1 h. This index increased more during the CGTT, especially in the case of analysis of blood from the pancreatic vein (Fig. 3).

The dynamics of the blood insulin concentration in the pancreatic vein reflects insulin secretion by the pancreas more accurately than the change in its concentration in blood from the peripheral vessels and the CGTT is a more sensitive test for determination of the incretory function of the pancreas than the standard GTT.

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EFFECT OF DISTURBANCE OF THE INNERVATION OF THE LIVER AND LOSS OF BILE ON BILIARY AND HEPATIC ENZYME ACTIVITY

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Continuous loss of bile from rats with a bile reservoir connected to the common bile duct led to an increase in specific activity of malate, lactate, glutamate, and glucose-6-phosphate dehydrogenases, alkaline and acid phosphatases, urokinase, and histidase in liver homogenates by the seventh day. By the tenth day their specific activity had fallen. After disturbance of the innervation of the rats' livers the ATP concentration fell sharply and the specific activity of the above-mentioned enzymes in the liver was considerably inhibited. During continuous loss of bile, fluctuating changes took place in the specific activity of these enzymes and also of sorbitol dehydrogenase in the bile, starting from the first and continuing until the tenth day of the experiment. Support for the view that these fluctuations were under the control of the nervous system was given by the considerable changes in their character following disturbance of the hepatic innervation.

KEY WORDS: enzymes; liver; disturbance of innervation; loss of bile.

The trophic influence of the nervous system is often manifested more clearly and distinctively when the function of an organ is disturbed. For the liver, one such model of this state is the continuous and prolonged loss of bile. Whereas biochemical indices of the liver have been studied in sufficient depth during the action of various factors of the nervous system, despite the theoretical and clinical importance of prolonged loss of bile and disturbance of the innervation of the liver, no information could be found on the biliary enzymes in these states. Moreover, the biliary enzymes have received little study even in healthy man and animals. Enzymes of the liver likewise have not been studied during loss of bile when the innervation of the organ is disturbed.

The object of this investigation was to determine the enzymes of the liver and bile during a disturbance of innervation of the liver and during loss of bile.

EXPERIMENTAL METHOD

The experiments of series I (duration 10 days) were carried out on 63 Wistar rats with a reservoir connected to the common bile duct for the continuous collection of bile [4]. The experiments of series II (the same duration) were carried out on 24 Wistar rats in which a receiver was connected to the bile duct immediately after disturbance of the innervation of the liver [6]. The specific activity of the following enzymes was determined daily in the bile and on the first, third, fourth, fifth, seventh, and tenth days of the experiments in liver homogenates during loss of bile and on the first, third, and seventh days after disturbance of innervation, by means of the SF-4A spectrophotometer: lactate dehydrogenase (LD), malate dehydrogenase (MD), glucose-6-phosphate dehydrogenase (G6PD), as described previously [7], glutamate dehydrogenase (GD) [14], sorbitol dehydrogenase (SD) [12], alkaline phosphatase

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