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(Citation)
The Kobe journal of the medical sciences,35(2):121-130

(Issue Date)
1989-04

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/E0035244

Kobe J. Med. Sci. 35, 121/130, April 1989

EFFECT OF HYPERGLYCEMIA ON HEPATIC EXTRACTION OF INSULIN IN TOTALLY PANCREATECTOMIZED DOGS

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INDEXING WORDS

hepatic insulin extraction; glucose clamp; insulin clearance; portal insulin infusion; pancreatectomized dogs

SYNOPSIS

Using the glucose clamp technique, we investigated the effect of blood glucose levels on hepatic extraction of insulin in pancreatectomized dogs.

A primed continuous infusion of regular insulin was administerd at a rate 1.5 mU/kg body wt./min into the portal or peripheral vein and the blood glucose level was clamped at hyperglycemia (over 200 mg/dl) or euglycemia (below 100 mg/dl) by a variable infusion of glucose through the saphenous or portal vein. Plasma insulin concentrations were measured during portal or peripheral insulin infusion, as were the metabolic clearance rates of insulin during insulin infusion via each route. Hepatic extraction of insulin was calculated from the two metabolic clearance rates using the insulin kinetic model (Pilo' method).

Received for publication : March 6, 1989
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Hepatic extraction of insulin during the hyperglycemic clamp was 45.8 ± 2.5%, significantly higher than that during the euglycemic clamp (35.7 ± 2.8% : $p < 0.01$).

When glucose was infused into the portal instead of the peripheral vein during the euglycemic clamp, hepatic extraction of insulin increased (44.7 ± 3.4 vs 37.6 ± 2.9% : $p < 0.05$).

It was concluded that hyperglycemia icreases the hepatic extraction of insulin. Moreover, portal hyperglycemia during the euglycemic clamp also increases the hepatic extraction of insulin. It is suggested that portal glucose concentration may affect the hepatic extraction of insulin.

INTRODUCTION

The anatomic relationship between the pancreas and the liver mandates that all of the insulin secreted by the pancreatic islets must traverse the liver before reaching the general circulation. Approximately 50% of the insulin is removed by that organ in a single transhepatic passage.[1)-4)]

Hepatic extraction largely controls peripheral insulin concentration and carbohydrate metabolism. Few workers have studied this extraction during intravenous glucose administration in animals[3,5,6)] because so much controversy remains concerning its regulation during stimulated insulin release.[7)] Further these studies employed glucose or insulin concentrations that were not in steady state.

This study employed steady states of both glucose and insulin concentrations using the glucose clamp method[8)] and pancreatectomized dogs.

The purpose of the present investigation was to clarify whether the hepatic extraction of insulin is influenced by glucose.

METHODS

Preparation of experimental animals

Twenty-one mongrel dogs weighing between 10 and 15 kg underwent total pancreatectomies under general anesthesia (sodium pentobarbital : 30 mg/kg).

At the time of operation micropore siliconized plastic cannulas were inserted. The cannula for the infusion of insulin or glucose was inserted through a tributary of the splenic vein and advanced until its tip was inserted in the portal vein, just below the bifirucation. Two cannulas for the infusion of 10% glucose and for measuring blood glucose concentration continuously with a glucose monitor (Kyoto Daiichi Kagaku, Japan) were inserted into the left and right jugular vein. One cannula for blood sampling was inserted into the saphenous vein.

After pancreatectomy, five enteric-coated tablets of digestive enzymes (Festal, Hoechst, Germany) were given with the daily food. The dogs were treated with monotard porcine insulin (Novo, Denmark) subcutaneously in the morning. Porcine insulin was chosen because it dose not induce the formation of antibodies for at least 2 months, and therefore plasma insulin can be measured accurately.[8] Insulin doses were between 15 and 25 U adjusted daily according to the degree of glycosuria as measured by Tes-Tape (Lilly, Shionogi, Japan), and the blood glucose as measured with glucosemeter (Kyoto Daiichi Kagaku, Japan).

Experimental design

All experiments were performed at two weeks after the operation. The pancreatectomized dogs were deprived of insulin for two days before each experiment to provide total insulin defficiency. The animals were under general anesthesia (sodium pentobarbital : 30 mg/kg). The leg with the blood sampling cannula was maintained in a heating box to ensure arterialization of the venous sample.[9] Each animal was studied on two occasions within a one-week period.

The expriment had two parts : 1) hyperglycemic and 2) euglycemic clamp studies. A primed continuous infusion of insulin (Human Actrapid, Novo, Denmark) was administered at rate of 1.5 mU/kg body wt./min. into the portal or peripheral vein during the glucose clamp.

1) Hyperglycemic clamp studies

During the first 60 or 80 min. glucose was not infused,

allowing the basal glucose concentration to fall toward 200 mg/dl. After attainning this plasma glucose cocentration, a variable infusion of glucose was started to maintain plasma glucose within ±10% of 200 mg/dl for 90 min.

2) Euglycemic clamp studies

After the hyperglycemic clamp, the glucose infusion was stopped, allowing plasma glucose to fall below 100 mg/dl. This concentration was maintained during the subsequent 90 min. by a variable infusion of glucose into i) the peripheral vein or ii) the portal vein (i.e., portal hyperglycemia). Plasma glucose concentrations in 10 animals did not fall to 100 mg/dl within 60 min. after the disconcentration of the glucose infusion, and these data were not included in the subsequent comparisons.

Blood samples for the measurement of peripheral immunoreactive insulin (IRI) concentrations were obtained at 0, 30, 60, 75 and 90 min. during each glucose clamp study.

The metabolic clearance rate of insulin (MCR) during portal and peripheral insulin infusion was calculated from the constant insulin infusion rate divided by mean plasma IRI concentrations at the last three points (60, 75 and 90 min.). Hepatic extraction of insulin (HE) was calculated according to Pilo's method[10] as follows;

$$HE\ (\%) = (MCR_{po} - MCR_{pe}) \,/\, MCR_{po} \times 100$$

MCR_{po} and MCR_{pe} represent MCR during portal and peripheral infusion of insulin, respectively. Using this model permits the calculation of the hepatic extraction of insulin without knowing the hepatic blood flow or insulin concentration in the hepatic artery or portal vein.

Analysis and statistical methods

Plasma glucose was measured continuously with the glucose monitor.[11] Plasma IRI was assayed by a double antibody method.[12] All data are expressed as means ± SE. The paired t-test was used to compare groups. A p value of < 0.05 was considered significant.

RESULTS

Plasma glucose and insulin concentrations during insulin infusion (Fig. 1)

The mean fasting plasma glucose and insulin concentrations were 325.3 ± 13.4 mg/dl and 5.3 ± 0.3 μU/ml, respectively.

In the hyperglycemic clamp study, the fasting plasma glucose concentration was lowered and maintained at 226.5 ± 9.1 mg/dl during the last 30 min. of portal insulin infusions and at 218.5 ± 10.3 mg/dl during the last 30 min. of peripheral insulin infusions.

The plsma insulin concentration was raised and maintained at steady state levels of 46.9 ± 2.9 μU/ml during the portal insulin infusion and 87.6 ± 6.3 μU/ml during the peripheral insulin infusion.

In the euglycemic clamp study, the fasting plasma glucose concentration was lowered to 90.6 ± 2.9 mg/dl during the portal and 92.0 ± 2.7 mg/dl during the peripheral insulin infusion.

The plasma insulin concentration was maintained at steady state levels of 54.3 ± 3.0 μU/ml during the portal and 91.6 ± 7.4 μU/ml during the peripheral insulin infusion.

There were no different plasma glucose levels between during the portal and the peripheral insulin infusion.

Metabolic clearance rates and hepatic extraction of insulin (Fig. 2)

In the hyperglycemic clamp study, the MCR_{pe} was 19.4 ± 1.4 ml/kg/min. and did not differ from that in the euglycemic study.

On the other hand, the MCR_{po} in the hyperglycemic clamp study (33.9 ± 3.9 ml/kg/min.) was significantly higher than that in the euglycemic clamp study (28.8 ± 1.8 ml/kg/min.; $p < 0.05$).

Both in the hyperglycemic and euglycemic clamp study, the MCR_{po} was significantly higher than the MCR_{pe} ($P < 0.05$).

Thus hepatic extraction of insulin during the hyperglycemia (45.8 ± 2.5%) was siginificantly higher than that during the euglycemia (35.7 ± 2.8%; $p < 0.01$).

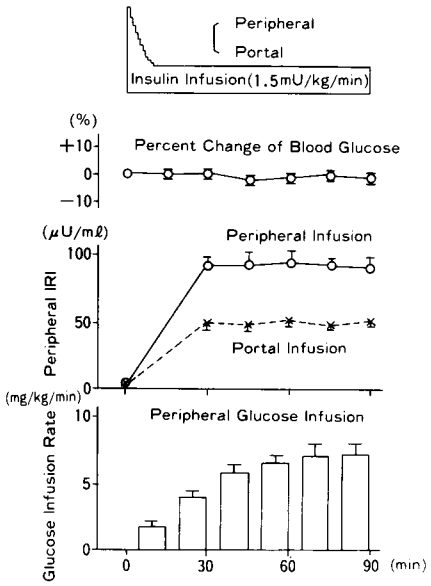

Fig. 1
Percent changes of blood glucose, peripheral serum insulin levels and glucose infusion rate during glucose clamp studies with peripheral and intraportal insulin infusion in depancreatized dogs.

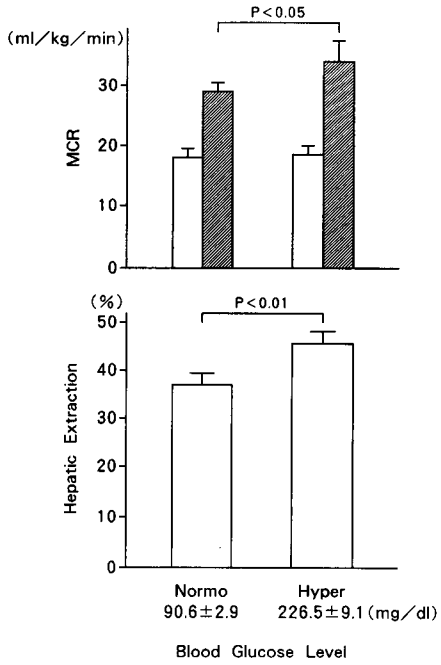

Fig. 2
Effect of blood glucose levels on metabolic clearance rate (MCR) and hepatic extraction of insulin. MCR with peripheral (white column) and intraportal (shadow column) insulin infusion.

Effect of portal hyperglycemia on the hepatic extraction of insulin (Fig. 3, 4)

When the plasma glucose was maintained at steady state levels under 100 mg/dl (90.3 ± 3.6 mg/dl) by a variable infusion of glucose (9.8 ± 0.9 mg/kg/min.) into the portal vein instead of the peripheral vein in the euglycemic clamp study, the MCR_{po} was significantly raised (at 33.4 ± 3.1 ml/kg/min.) compared with that of peripheral vein (29.1 ± 1.7 mg/kg/min.; $p < 0.01$), although the MCR_{pe} remained unchanged.

The hepatic extraction of insulin during portal glucose infusion (44.7 ± 3.4%) was higher than that (37.6 ± 2.9%; $p < 0.05$) during peripheral glucose infusion in euglycemia.

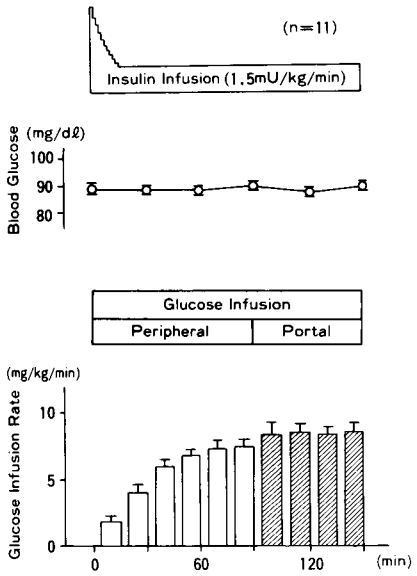

Fig. 3
The scheme of euglycemic clamp studies with peripheral and intraportal glucose administration.

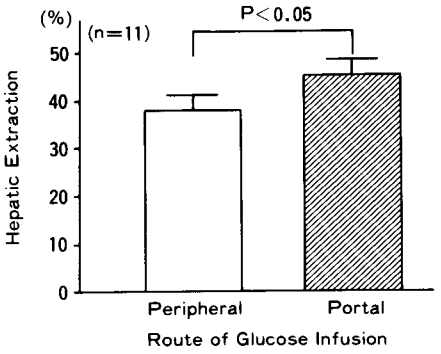

Fig. 4
Effect of glucose infusion route on hepatic extraction of insulin during euglycemic glucose clamp.

DISCUSSION

In the present study, we observed that hyperglycemia caused more hepatic extraction of insulin than euglycemia.

Studies with oral glucose administration and intravenous glucose administration have showed reduction,[7,13)-15)] no change,[6,16,17)] or an increase[5,8)] in hepatic extraction of insulin. The reason for these discrepancies may be the different experimental techniques and species difference.

In this study, we employed the glucose clamp technique, because the steady state of glucose and insulin levels were needed to see true hepatic extraction of insulin. The balance studies which are calculated using the amount of insulin presenting to and

leaving from the liver may be difficult to interpret when the concentration of insulin changes with each transhepatic passage in non-steady-states of insulin and glucose. In particular, the time delays and intra-organ distribution may distort the relationship between input and output.

The increase of hepatic extraction of insulin under hyperglycemic conditions could be attributed to the increase of MCR_{po} but not the change of MCR_{pe}. MCR_{po} is a combined measurement of hepatic uptake of passing and recirculating insulin, and subsequent removal in the peripheral tissue. The major difference between the results of portal and peripheral infusions is due to the amount of insulin removed during first passage after portal infusion. The reason MCR_{pe} remained unchanged might be that the amount of insulin diverted to the liver was comparatively small and masked by uptake in other tissues. Another possibility is that the change in hepatic extraction may be only demonstrated when the insulin concentration in the portal vein in higher than that in the peripheral circulation as seen in our body. If so, our data may be underestimations because the hepatic extraction of insulin was assumed to occur at the same rate during portal and peripheral infusion of insulin. Further studies should be carried out.

The increase of hepatic extraction under hyperglycemic condition is not clear. However, portal glucose concentration in the liver might be a more important factor in the modulation of the hepatic extraction of insulin, as hepatic extraction of insulin during euglycemia with portal hyperglycemia induced by glucose infusion into portal vein also increased.

In conclusion we demonstrated that hyperglycemia produced an increase in hepatic extraction of insulin compared with that during euglycemia during a steady state of glucose and insulin concentrations using the glucose clamp method. Moreover, portal hyperglycemia during the euglycemic clamp in peripheral circulation also increased the hepatic extraction of insulin. The increase under these conditions resulted from the increase of MCR_{po} without any change of MCR_{pe}. It is suggested that hyperglycemia, especially in portal vein, is an important factor in the modulation of the heptaic extraction of insulin, and which is usually seen in the postrandial rise of glucose, as one of the glucose regulation.

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