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Rates of Blood Glucose Appearance and Disappearance during Hyperglycemia Induced by Alloxan in Sheep

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Summary

Alloxan (75 mg/kg body weight) was administrated intravenously to three shorn sheep to investigate glucose metabolism in the diabetic state. The concentrations of plasma glucose, 3-hydroxybutyrate, free fatty acids and insulin were measured for 52 hrs after the injection of alloxan. Rates of blood glucose appearance and disappearance were measured during the first 6 hrs and at 2 days after the injection of alloxan using isotope dilution methods. The concentrations of plasma glucose, 3-hydroxybutyrate and free fatty acids increased gradually but markedly and reached stable values 46 hrs after the injection. The plasma insulin level was unchanged at 5 hrs, but decreased to less than 10 per cent of the preinjection level at 2 days after the injection. On the other hand, both the rates of appearance and disappearance of blood glucose increased markedly. The appearance rate reached a maximal value faster than the disappearance rate. These results suggest that hyperglycemia following alloxan administration is caused by a rapid acceleration of gluconeogenesis and glycogenolysis occurring before insulin deficiency is established.

In ruminants, a large proportion of energy requirements is supplied as volatile fatty acids, which are produced by the fermentation of cellulose and starch by the rumen microorganisms and are absorbed through the digestive tract epithelium. Since only small quantities of glucose are absorbed from the alimentary tract in ruminant, glucose utilization in the whole-body must depend on glucose production by gluconeogenesis in the liver and kidney (1).

Insulin and glucagon, secreted from the islets of Langerhans in the pancreas, play a central role in the control of glucose metabolism in ruminant animals as in nonruminant species (2). Insulin accelerates glucose utilization in muscle and

adipose tissues and stimulates hepatic glucose phosphorylation and glycogen synthesis. Glucagon, on the other hand, stimulates hepatic glycogenolysis and gluconeogenesis. Alloxan is known to produce an experimental hyperglycemia by preventing insulin production in the pancreatic B cell. A previous report from our laboratory revealed that the intravenous injection of alloxan (75 mg/kg body weight) caused severe hyperglycemia and glucosuria, and resulted in marked increase in plasma free fatty acids and ketone bodies in sheep (3).

Although the turnover rate of blood glucose is commonly measured under steady state conditions, as indicated by a stable plasma glucose level, it can be also measured using Steele's equations during non-steady states such as following administrations of glucose (4), catecholamines (5), glucagon (5) and insulin (6, 7). The objective of this experiment was to investigate the mechanism of hyperglycemia by determination of the turnover of blood glucose in alloxanized sheep.

Materials and Methods

Animals

Three adult Corridale wethers weighing about 50 kg were used. The animals were kept in metabolic cages, and fed lucerne hay cube and commercial concentrates (each at 10 g per kg body weight) once daily at 9 : 00. Water was given *ad libitum*. Polyethylene catheters were inserted into both jugular veins at least a day before blood sampling commenced.

Experimental Procedure

Alloxan (75 mg/kg body weight) dissolved in 50 ml of 0.9 per cent NaCl solution was injected through the jugular catheter over 2 min. Blood samples were obtained from another jugular catheter before and after the injection of alloxan. [U-^{14}C]Glucose was continuously infused (0.25 μCi/min at a concentration of 0.5 μCi/ml of saline) immediately after a priming injection (25 μCi in 10 ml of saline) was given into the jugular vein in order to measure rates of blood glucose appearance and disappearance. Venous blood samples (5 ml) were taken from another catheter at intervals of 30 and 60 min during the infusion period. Blood samples were centrifuged at 0°C, and plasma was frozen at −20°C until analysis.

Analytical Methods

The concentrations of plasma glucose and 3-hydroxybutyrate were enzymically determined (8, 9), while that of plasma free fatty acids was determined by a NEFA-test kit (Wako, Osaka, Japan). Plasma insulin was assayed as described by Sasaki and Takahashi (10). The isolation of glucose from plasma was as described previously (11), and the radioactivity of plasma glucose was measured by liquid scintillation counting (Packard, Model 3385, USA). Blood glucose

kinetics were calculated by the equations (12) originally described by Steele (13).

Results and Discussion

1) *Effect of Alloxan Administration on Plasma Constituents*

Changes in the concentrations of plasma glucose, free fatty acids, 3-hydroxybutyrate and insulin before and after the injection of alloxan are shown in Fig. 1. Plasma glucose increased rapidly from 63 to 270 mg/dl during the first 3 hrs after the injection. Plasma glucose decreased transiently to 200 mg/dl thereafter, but increased again to 400 mg/dl at 46 hrs after the injection. Plasma free fatty acids increased gradually from 0.4 mEq/l to 1.3 mEq/l. Plasma 3-hydroxybutyrate increased slightly during the first 24 hrs, and thereafter increased markedly to about 40 mg/dl at 46 hrs after the injection. These results are very similar to the data reported by Hidari *et al.* (3). The plasma insulin level was initially 11 μU/ml, and this level was still maintained at 5 hrs, but the concentration decreased markedly to 1 μU/ml at 48 hrs after the injection of alloxan.

2) *Effect of Alloxan Administration on Blood Glucose Metabolism*

It is well known that alloxan inhibits insulin secretion by preventing insulin production in the pancreatic B cells. It is likely that the insulin deficiency induced by alloxan injection causes a decrease in peripheral glucose utilization as

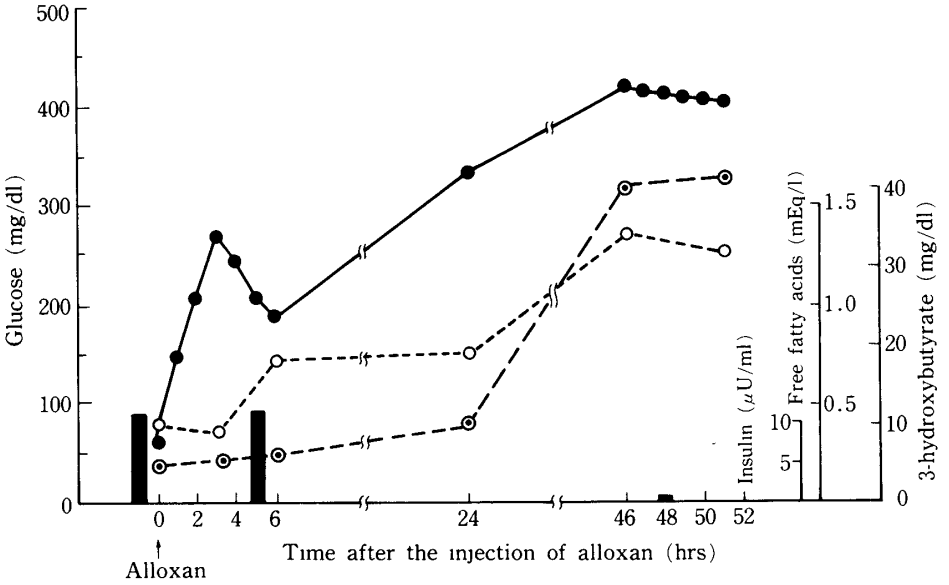

FIG. 1. The concentration of plasma glucose (●), free fatty acids (○), 3-hydroxybutyrate (⊙) and insulin (solid bars)before and after the intravenous injecion of alloxan (75 mg/kg body weight). Data are expressed as the mean from three sheep.

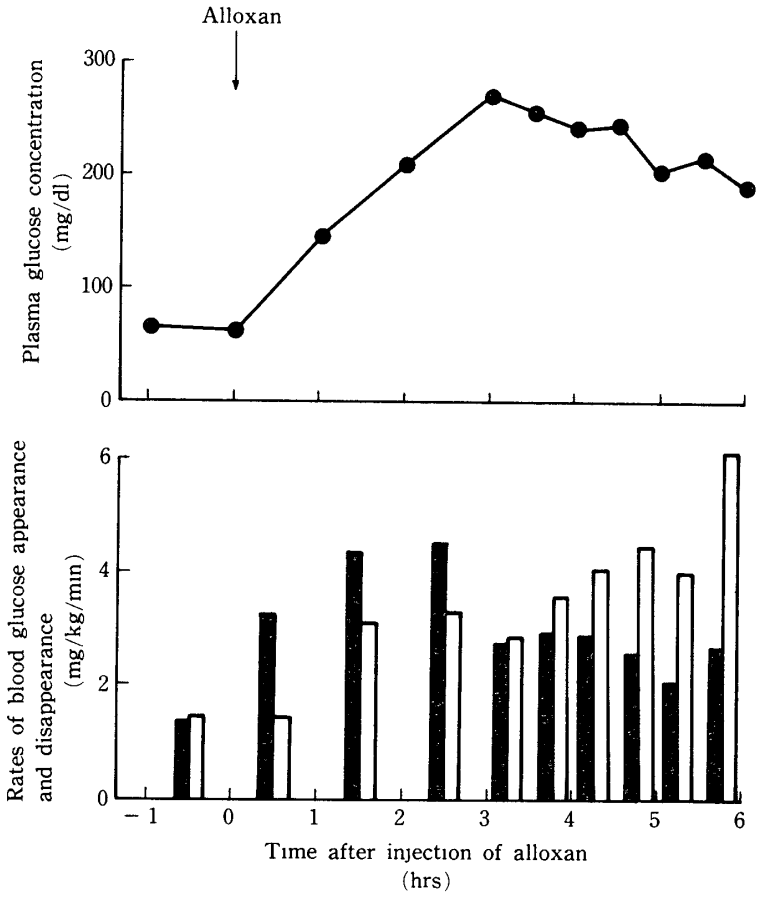

FIG. 2. Plasma glucose concentration and rates of blood glucose appearance (solid bars) and disappearance (open bars) before and after the intravenous injection of alloxan (75 mg/kg body weight). Data are expressed as the mean from three sheep.

well as accelerated rates of lipolysis and proteolysis, which consequently result in an increase in the blood glucose concentration.

In the present experiment, the rates of appearance and disappearance of blood glucose were measured during both the initial changing period and the later plateau period of plasma glucose levels. Appearance and disappearance rates before the alloxan injection was almost similar (1.4-1.5 mg/kg/min). The appearance rate of blood glucose increased markedly, more than doubling within 1 hr after the injection of alloxan, and tended to decrease gradually thereafter (Fig. 2). On the other hand, the disappearance rate of blood glucose increased more gradually, and reached a maximal value (5.6 mg/kg/min) at 6 hrs after the injection. The rate of appearance was exceeded by the disappearance rate by 3 hrs after injection. It is likely that the marked increase in the rate of appearance of blood glucose after the injection of alloxan was responsible for the marked increase in plasma glucose concentrations immediately after the alloxan injection. The marked increase in the rate of disappearance of blood glucose 3 hrs after the

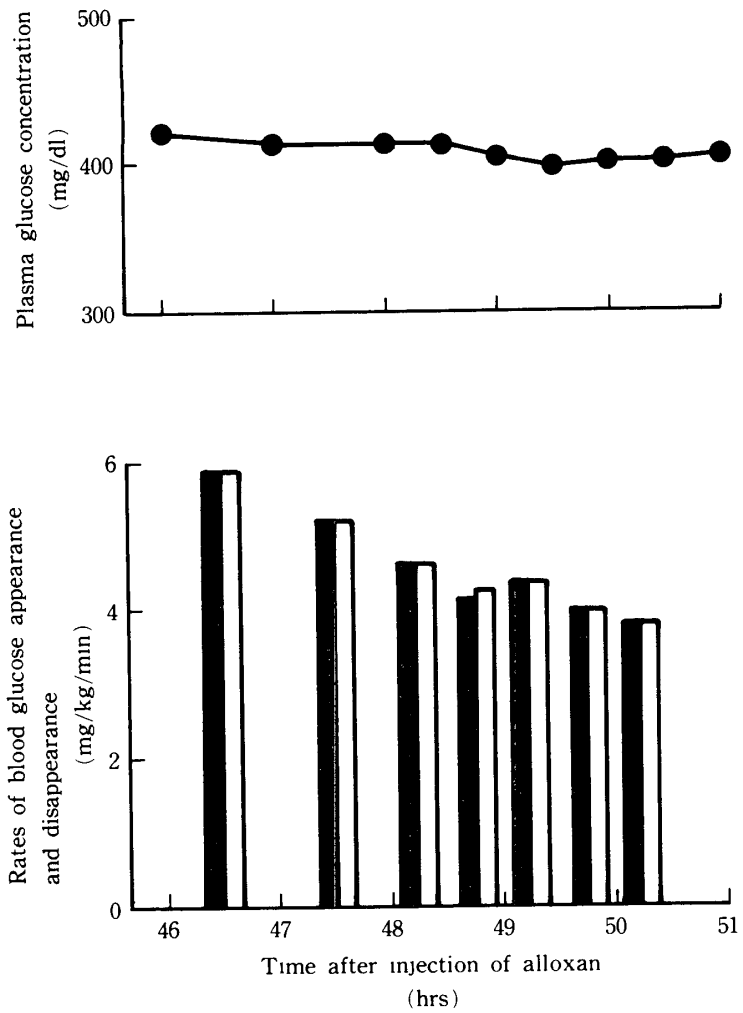

FIG. 3. Plasma glucose concentration and rates of blood glucose appearance (solid bars) and disappearance (open bars) 2 days after the intravenous injection of alloxan (75 mg/kg body weight). Data are expressed as the mean from three sheep.

alloxan injection accompanied by a decline in the appearance rate would influence the blood glucose concentration resulting in the establishment of a stable level. In the present experiment, the measured disappearance rate does not neccessarily imply an increase in tissue utilization of blood glucose, because glucose would be excreted into the urine when the concentration of blood glucose increased above the renal threshold for glucose.

The increased rate of blood glucose appearance immediately after the injection of alloxan may be caused by changes in other hormonal and neural factors rather than by insulin deficiency, because the plasma insulin level was unchanged at that time. Glucagon could be one of the important hormones, because Cherrington *et al.* (14), who measured glucose production rates during the combined infusion of somatostain and glucagon in dogs, found that glucose production

increased markedly initially, and then decreased gradually to the preinjection level. Furthermore, it has been reported that diabetes causes an absolute or relative hyperglucagonemia (15), although it is generally accepted that hyperglycemia inhibits and hypoglycemia accelerates glucagon secretion (16). In this regard, Ohneda (17) concluded that high plasma glucagon levels play an important role in diabetic disorders, and he suggested that not only insulin deficiency but also hyperglucagonemia is implicated in the development of diabetes. Plasma glucagon concentrations were, unfortunately, not measured in the present experiment.

The rates of blood glucose appearance and disappearance at 2 days after the injection of alloxan are shown in Fig. 3. Over this period the enhanced rates of blood glucose appearance and disappearance decreased gradually, though the elevated plasma glucose levels were almost constant. In previous reports (18,19), a plateau of specific activity of plasma glucose was obtained during 3 hrs after the primed infusion of [U-^{14}C]glucose in normal sheep. However, the possibility that the specific activity of plasma glucose did not reach a plateau level in alloxanized sheep in the present experiment cannot be excluded, if glucose metabolism in alloxanized sheep differs from that in normal sheep.

It is surprising that the rate of blood glucose appearance after the injection of alloxan was maintained at about 2 times larger than the preinjection value. In this regard, it has been suggested that diabetic animals maintained membrane permeability and intracellular metabolism of glucose by inducing hyperglycemia when intracellular glucose metabolism was inhibited by insulin deficiency (20). Moreover, Krebs (21) described an increase in gluconeogenesis from amino acids and glycerol and a lack of intramitochondrial oxaloacetate which was neccessary for hepatic oxidation of free fatty acids mobilized from adipose tissue, resulting in an increase in ketogenesis. These reports suggest that hepatic and renal gluconeogenesis and glycogenolysis might be accelerated in animals treated with alloxan. Therefore, it is likely that hyperglycemia might be a positive adaptation, although not an ideal state, allowing diabetic animals to compensate for the decrease in glucose utilization by peripheral tissues induced by insulin deficiency.

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