Effect of oleic acid and palmitic acid on glucose and insulin clearance in the perfused rat hindquarter

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Elevated free fatty acid levels are frequently associated with insulin resistant state,such as diabetes mellitus, fasting and obesity[1-3]. Svedberg et al[4,5] recently reported that fatty acids in physiological concentrations inhibited insulin binding, degradation and function in isolated rat hepatocytes and perfused rat liver, apparently dependent on the oxidation of the fatty acids. This may be one of causes for insulin resistance associated with elevated FFA levels. It is well known that the decrease in muscle glucose clearance is involved in the insulin resiatance. To date, there have been conflicting results regarding the effects of fatty acids on the muscular glucose clearance, and the effects of fatty acids on the muscular insulin clearance have remained to be elucidated. In the present study, we investigated the effects of oleic acid and palmitic acid on the clearance of glucose and insulin in perfused hindquarter of rats.

Materials and methods

Animals

Female Wistar albino rats weighing approximately 150g were used in the present study.

Reagents

Dextran T-70 was purchased from Green Cross Co., Osaka, Japan. Bovine serum albumin (BSA), oleic acid, and palmitic acid were obtained from Sigma Chemical Co., St Louis, MO.

Perfusion of hindquarter

The modified method[6] of Ruderman et al[7] was used for the isolation and perfusion of the rat hindquarter. In brief, the abdomen was opened after anesthesia. After intravenous-injection of heparin (200U), the upper portion of the abdominal aorta was ligated, and then the abdominal aorta was incised at the point between the left renal and the iliolumbar vessels. An inflow cannula was inserted and passed to a point midway between the iliolumbar vessels and the aortic bifurcation. The cannula was then fixed in place. Then the perfusion pump was started. All viscera except the urinary bladder, testes, prostate and seminal vesicle were removed, and several abdominal branches of great vessels were ligated. Since

it was not possible to collect the perfusate quantitatively from the inferior vena cava, because of anastomotic connections with the vertebral veins, the operated animal was bisected just above the aortic cannulation. The effluent was allowed to drip into the chilled tube. The hindquarter was perfused without recirculation with a synthetic medium at a flow rate of 0.5 ml/min/g muscle weight.

Perfusion medium

The perfusion medium consisted of a Krebs-Ringer bicarbonate buffer containing 0.5% BSA, 4.6% Dextran T-70, and 8.3 mM glucose. To prevent glucose metabolism by erythrocytes, the erythrocyte-free medium was used in the present study.

Porcine insulin (crystalline, glucagon-free, Eli Lilly Co., Indianapolis, IN) was added at concentrations given for experiment. Oleic acid and palmitic acid were mixed to albumin to give the indicated concentrations in the perfusion medium.

Perfusion method

The hindquarter was perfused with the synthetic medium containing 500 μM oleic acid and 250 μM palmitic acid or 1,000 μM oleic acid and 500 μM palmitic acid in the presence or absence of 500 μU/ml insulin. After an equilibration period for 20 min, the venous effluent was collected every 5 min for 30 min, and stored at -20 ℃ until the time of assay. The medium and the hindquarter were kept at 37 ℃ during perfusion, and the medium was kept bubbling with a mixture of 95% O_2 and 5% CO_2. The pH was maintained at 7.4.

Calculations

The oxygen consumption in the hindquarter was calculated from the difference of quantities between infused and effused.

The clearance rate of glucose or insulin by the hindquarter for 30 min was calculated by a formula : (Glucose or insulin infused for 30 min−glucose or insulin effused for 30 min) $\times$ 100 (%)/glucose or insulin infused for 30 min.

Measurements

The oxygen content of the medium was determined according to VanSlyke and Neill[8]. Effluent glucose concentration was measured by a glucose oxidase method[9]. Lactate concentrations were measured enzymatically[10]. ATP was determined by the method of Lamprecht & Trautschold[11]. Insulin was measured by radioimmunoassay[12].

Statistical evaluations

The data are expressed as means ± SD. Analysis of variance and two-tailed Student's *t* test were used for statistical evaluations.

Results

Oxygen consumption and formation of lactate and ATP

Oxygen consumption was 0.3 to 0.4 μmol O_2 /min/g, and lactate formation was 0.09 to 0.12 μmol/min/g in perfused hindquarter. ATP concentration in perfused hindquarter (4.5 ± 0.5 μmol/g) was not significantly different from that in the hindquarter in vivo (5.0 ± 0.5 μmol/g).

Glucose clearance by the perfused hindquarter

As shown in Table 1, though glucose clearance in the hindquarter perfused without insulin was faintly decreased by the addition of fatty acids, it with 500 μU/ml insulin was

Significantly decreased by the addition of 1, 000 μM oleic acid and 500 μM palmitic acid.

Table 1 Glucose and insulin uptake by the perfused hindquarter of rats

	Insulin (−)	Insulin (500 μU/ml)	
	Glucose clearance	Glucose clearance	Insulin clearance
FFA (−) (n=6)	12.7 ± 3.8 %	16.9 ± 4.1 %	11.0 ± 3.9 %
Oleic acid (500 μM) (n=6) Palmitic acid (250 μM)	12.0 ± 3.3 %	14.3 ± 3.0 %	8.9 ± 4.1 %
Oleic acid (1,000 μM) (n=6) Palmitic acid (500 μM)	10.5 ± 3.2 %	12.0 ± 3.0 %*	7.6 ± 4.4 %

$p<0.05$, significantly different from FFA (−) group.

Insulin clearance by the perfused hindquarter

Although insulin clearance in the perfused hindquarter was not significantly decreased by the addition of fatty acids, the fall in insulin clearance is of similar magnitude to the fall in glucose disposal.

Discussion

The data of oxygen consumption and formation of lactate and ATP by the perfused hindquarter showed that this preparation functions well both in terms of organ integrity and gas exchange over the time period used in the experiments. Recently, we have reported that oleic acid and palmitic acid were the major components of increased serum fatty acids in diabetes mellitus[13]. Therefore, in the present study, the effect of oleic acid and palmitic acid on glucose clearance was investigated in the perfused rat hindquarter. In the absence of insulin, fatty acids did not influence glucose clearance, while in the presence of insulin, glucose clearance was significantly decreased by the addition of higher concentrations of fatty acids. If fatty acids inhibit non-insulin dependent glucose clearance, glucose clearance in the hindquarter may be impaired in the absence of insulin. Non-insulin-dependent glucose clearance may not be impaired by fatty acids. I believe this is the first report showing that fatty acids directly inhibit the insulin-dependent glucose clearance in the muscle. The fall in insulin uptake rate, though not significantly, is of similar magnitude to the fall in glucose uptake rate, suggesting that an inhibitory effect of fatty acids on muscular glucose uptake may be due to the decrease in insulin binding. Although the mechanism of this phenomenon remains to be clarified and further studies are required to resolve this molecular force, the present results were consistent with the report of Svedberg et al[4, 5] that fatty acids, at physiological concentrations, inhibited insulin binding and degradation in isolated rat hepatocytes and perfused rat liver.

In summary, we conclude that oleic acid and palmitic acid, at physiological concentrations, directly inhibit the muscular uptake of insulin and glucose as well as the hepatic uptake of insulin and glucose.

Summary

Oleic acid and palmitic acid were applied to elucidate the effects on the clearance of glucose and insulin in the muscle of perfused hindquarter of rats. In the absence of insulin, glucose clearance in the hindquarter was faintly decreased by the addition of fatty acids. In the presence of 500 μU/ml insulin, glucose clearance in the hindquarter was significantly decreased by the addition of 1,000 μM oleic acid and 500 μM palmitic acid. Although insulin clearance in the perfused hindquarter was not significantly decreased by the addition of fatty acids, the fall in insulin uptake rate is of similar magnitude to the fall in glucose uptake.

These results indicate that fatty acids directly inhibit glucose clearance by the muscle probably through the decrease in insulin binding, and that fatty acids may directly be responsible for the decrease in peripheral insulin sensitivity.

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