EFFECT OF LACTATE ON INSULIN ACTION IN RATS

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Abstract

The aim of the study was to explore the effect of lactate on insulin-stimulated glucose uptake in rats. Thirty Wistar rats, weighing 250 – 300 g. were arbitrarily divided into one of three groups (n =10): insulin (1 IU/kg) treated group, lactate (80 mg/kg), and insulin plus lactate treated groups. Blood glucose levels were measured in venous samples collected from the tail vein over 3 hour period after insulin or/and lactate administration in 30-minute intervals.

To estimate the influence of lactate on insulin blood level, a total of 20 rats were divided into 4 groups (n = 5): saline, insulin, lactate, and insulin plus lactate treated group, respectively.

Sixty minutes after the appropriate application of the same doses of insulin, lactate, and lactate plus insulin, as in the previous part of the experiment, plasma insulin and blood glucose levels were determined in blood samples drawn from the abdominal aorta. Lactate in combination with insulin, in comparison to insulin application alone, caused a dramatic increase in plasma insulin level ($p<0,001$) and more profound hypoglicaemia ($p<0,001$). The results of this investigation indicate that lactate application significantly increases the rate of glucose uptake from peripheral blood caused by exogenous insulin action. The possible involvement of lactate in the mechanism of enhanced glucose uptake due to insulin action after physical exercise is discussed.

KEY WORDS: lactate, insulin, glucose uptake, rats.

Introduction

Exercise and diet still remain a cornerstone of type 2 diabetes therapy. In the past few years numerous studies have addressed this topic. These studies have had a profound influence on current views of the physical activity effects on muscle glucose uptake as well as insulin sensitivity and resistance (1). It has been shown that physical activity lowered the insulin dose required for half-maximum stimulation of glucose transport and utilization by skeletal

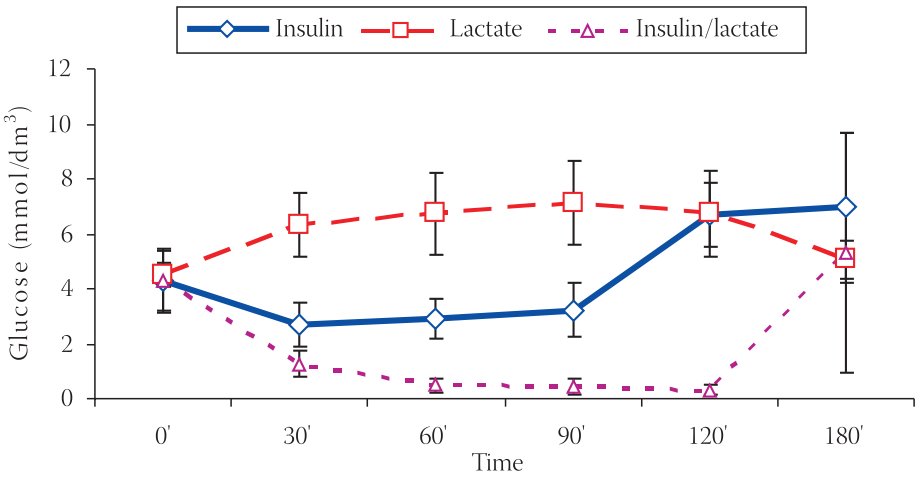
muscle, suggesting that at least one of the mechanisms is enhanced insulin sensitivity, and to a lesser degree glucose effectiveness (2). On the other hand, insulin sensitivity for glucose uptake decreases across the forearm in healthy people after a few days of continuous bed rest (3). Similarly, early morning insulin resistance is observed in insulin dependent diabetic patients (4). Also, it is noteworthy that lactate is the main metabolic product accumulated during muscle activity, which indicates its possible influence on insulin sensitivity and muscle glucose uptake. By contrast, some data indicate increased lactate concentration in obesity, which may play an important role in glucose transport and utilization, and deeply affect insulin sensitivity (5). Although the molecular mechanisms are yet unclear, it has been reported that the inhibition of glucose transport and suppression of insulin stimulated glycolysis, as well as decreased insulin stimulated glucose uptake is involved in this phenomenon. It is also known that lactate suppresses glycolysis rather than decreasing insulin stimulated glucose uptake. In addition, it has been shown that lactate induced insulin resistance is associated with impaired insulin signaling and decreased insulin stimulated glucose transport in skeletal muscle. Furthermore, it has been shown that elevated lactate plasma levels directly affect β-cells in the pancreas, inducing increased insulin secretion (6). Therefore, the purpose of this study was to find out whether lactate, which accumulation regularly follows muscle activity, has any influence on insulin action upon the rate of glucose uptake in rats.

Materials and Methods

Wistar rats of both sexes, weighing 250-300 grams, were used for all experiments. Rats were housed in individual cages in controlled environment with a 12-h light –dark cycle, and received a standard pellet diet and water, *ad libitum*. All experimental procedures were approved by the Institutional Animal Care and Use Committee. Thirty rats were arbitrarily divided into one of three groups of approximately the same body weight: 1. insulin treated, 2. lactate treated and 3. insulin plus lactate treated group. After an overnight fasting and collection of control blood samples, the rats in the first group were injected subcutaneously with insulin (1 IU/kg), rats from the group 2. intraperitoneally with lactate (82 mg/kg), while those from the group 3. received insulin subcutaneously and lactate intraperitoneally. The blood samples were collected from the tail vein before (0') and 30, 60, 90, 120 and 180 minutes after the respective treatment and analyzed for glucose. To investigate the effect of lactate, insulin, and insulin plus lactate treatments on plasma insulin and liver glycogen levels, four groups of rats (n = 5) were set up: control, insulin treated, lactate treated, and insulin plus lactate treated group. The control group was treated with an adequate volume of saline. Other groups were treated with insulin, lactate, and insulin plus lactate using the same doses as in the previous experiment. One hour after treatment, the animals were anaesthetized by ether and, after laparotomy, blood samples for measurement of blood glucose and plasma immunoreactive insulin were obtained via abdominal aorta puncture into heparinized syringes. Liver samples were taken for the assessment of liver glycogen level. Concentration of glucose in arterial blood and plasma was analyzed by O-toluidine method using kits purchased from "Kemika", Zagreb, Croatia. The insulin in plasma was determined radioimmunologically using human insulin as a standard. Glycogen level in liver tissue was determined by Best's carmine method. Standard statistical methods were used to analyze the data. All values are expressed as means ± SD. Statistical significance of differences was determined by Student´s test.

Results

The influence of lactate on insulin activity is presented as the rate of glucose uptake from peripheral blood. The blood levels of glucose during a 3- hour period after appropriate treatment are shown in Fig. 1. Obviously, lactate application enhanced the action of exogenous insulin. The mean values of blood glucose level after insulin/lactate application are lower in comparison to that in the group treated with insulin alone, and the differences were statistically significant ($p<0,001$).The application of lactate alone resulted in a significant increase in glucose blood levels ($p< 0,001$). The values of plasma insulin and blood glucose in rats 60 minutes after treatment with saline, insulin, lactate, and insulin/lactate are shown in Table 1. Lactate application decreased insulin plasma levels (76,6 pmol/dm^3) in comparison to the control group (99 pmol/dm^3), but the difference was not statistically significant. Glucose blood levels increased significantly ($p< 0,001$) due to lactate application. As expected, insulin application decreased glucose blood levels and increased insulin plasma levels. In comparison to the value in the control group, the differences were statistically significant ($p<0,001$). Insulin/lactate application increased insulin plasma level (2749,6 pmol/dm^3). In comparison to the values in the group treated only with insulin (765,4 pmol/dm^3), the difference was statistically



*The differences are statistically significant in comparison to the corresponding values in the insulin-treated group (p<0,001).

FIGURE 1. Mean values of glucose level (mmol/dm^3) in rats after treatment with insulin, lactate and insulin plus lactate.

significant (p<0,001). Glucose blood level (1,66 mmol/dm^3) was significantly decreased (p<0,001) due to insulin/lactate application in comparison to the value of the group treated with insulin (3,72 mmol/dm^3). Table 1. The main values of plasma insulin (pmol/dm^3) and blood glucose (mmol/dm^3) in rats after treatment with insulin, lactate and insulin plus lactate The liver glycogen content was approximately the same in insulin and insulin/lactate treated groups. Lactate application did not change blood pH values significantly.

Discussion

The results of our study in rats show that lactate injected simultaneously with insulin significantly increases insulin mediated uptake of glucose from peripheral blood (Figure 1). In our preliminary experiments, where the appropriate doses of lactate were tested, the higher doses of lactate in combination with insulin caused a profound decrease of blood glucose levels, with deaths of few animals as a consequence. In available literature we could not find any data concerning such an effect of lactate. However, there are some similarities among our findings and the observations of others that vigorous exercise stimulates glucose transport and utilization, and in this effect insulin may play a permissive role (7,8). This phenomenon is explained by enhancement of insulin sensitivity for glucose removal according to the finding that an increase of insulin sensitivity occurred after physical training in humans (9,10) and animals (11,12). At the same time it has been suggested that skeletal muscle rather than liver, is the organ primarily responsible for increased sensitivity to insulin-induced glucose uptake during exercise (12), which is in accordance with the results of our liver glycogen measurements. With regards to the observed increase of blood glucose level after the application of lactate alone, it is probably the consequence of increased gluconeogenesis. In this case our results are consistent with those of others. It has been suggested that lactate accomplishes this effect by suppressing insulin induced glycolysis rather than decreasing insulin-stimulated glucose uptake (6). However, Vettor R. et al. (5) have shown that peripheral glucose disposal was significantly reduced by lactate infusion. Additionally, they have shown that in conditions characterized by increased lactate plasma levels this end product of glucose metabolism could be utilized preferentially to satisfy glucose utilization. After all, these effects of lactate are still not completely clear, and require further investigation. Based on the previous findings that in both man (9,10,13,14) and animal (11,15) physical activity reduces

Plasma Parameter	Saline treated group (n=5)	Insulin treated group (n=5)	Lactate treated group (n=5)	Insulin+lactate treated group (n=5)
Plasma insulin (pmol/dm^3)	99 + 66,25	765,40+179,07*	76,60 + 55,72	2.749,60+1.477,89*
Blood glucose (mmol/dm^3)	4,62 + 0,52	3,72 + 0,64*	6,16 + 0,37*	1,66 +0,73*

*** The differences are statistically significant in comparison to the corresponding values in the saline treated group (p<0,001).**

TABLE 1. The main values of plasma insulin (pmol/dm^3) and blood glucose (mmol/dm^3) in rats after treatment with insulin, lactate and insulin plus lactate

basal serum insulin level, we measured plasma insulin levels in the control group (saline solution), insulin, lactate, and insulin/lactate treated rats. Contrary to the previous findings, our results (Table 1.) indicate that, in the case of simultaneous application of insulin and lactate, plasma insulin level are enormously increased. The difference between the intensity of the effects of physical activity and simultaneous application of insulin and lactate could be explained by the presence of exogenous insulin in our experiments. This could also be the reason behind decrease of plasma insulin level in the case of the application of lactate alone. Our findings on the effects of independent lactate application differ from the previous findings. Investigations carried out a few years ago, showed that increased blood lactate concentration increases insulin secretion (5,16). Attempts to explain this effect of lactate on insulin secretion by lactate action on K^+ and Ca^{++} channels have suggested that the lactate stimulates insulin secretion by suppression of Ca^{++} efflux from the cell without blocking K+ channels (16) . Right now, it is difficult to explain the effect of simultaneous application of insulin and lactate on plasma insulin level in our rats. It is well known that glucose and its metabolites are major determinants of insulin biosynthesis and secretion. However, a large number of physiological and pharmacological agents can also act as secretogogues (17,18). Therefore, it is possible to speculate that some products of lactate metabolism in islet cells of the pancreas, induced by exogenous insulin, may have this effect on insulin secretion.

Conclusion

Our present results provided more evidence of lactate effects on insulin promoted glucose uptake in rats. Exact evidence on the possible involvement of lactate in the mechanism of glucose uptake enhancement after physical activity and exercise, as well as an exact explanation of the observed lactate effects in this study, require more information and further investigation.

References

(1) Burant C.F., Lemmon S.K., Treutallar M.K., Buse M.G. Insulin resistance of denervated rats muscle: A model for impaired receptor-function coupling. Am. J. Physiol. 1984; 247:E657-E666.

(2) Mondon C.E., Dolkas C.B., Reaven G.M. Site of enhanced insulin sensitivity in exercise-trained rats at rest. Am. J. Physiol. 1980;239:E169-E177.

(3) Lipman R.L., Raskin P., Love T., Triebwasser J., Lecocq F.R., Schnure J.J. Glucose intolerance during decreased physical activity in man. Diabetes 1972;21:101-107.

(4) Bright J.M., Melton T.W., Rogol A.D., Clarke W.L. Failure of cortisol blockade to inhibit early morning increases in basal insulin requirements in fasting insulin-dependent diabetics. Diabetes 1980; 29:662-664.

(5) Richter E.A., Garretto L.O., Goodman M.N., Ruderman N.B. Muscle glucose metabolism following exercise in the rat. J. Clin. Invest. 1982;69:785-79.

(6) Choi C. S., Kim Y-B., Lee F.N., Zabolotny J.M.,Kahn B.B., Youn J.H. Lactate induces insulin resistance in skeletal muscle by suppressing glycolysis and impairing insulin signaling. Am. J. Physiol. Endocrinol. Metab. 2002;283:E233-E240.

(7) Berger M., Hagg S.A., Rufferman N.M. Glucose metabolism in perfused skeletal muscle: interaction of insulin and exercise on glucose uptake. Biochem. J. 1975;146:231-238.

(8) Ivy J.L., Holloszy J.O. Persistent increase in glucose uptake by rat skeletal muscle following exercise. Am. J. Physiol. 1981;241:200-203.

(9) Bjorntorp P., Fahlen M., Grimby G., Gustafson A., Holm I., Renstorp P., Schernsten T. Carbohydrate and lipid metabolism in middle-aged physically well-trained man. Metabolism 1972; 21:1037-1044.

(10) Lipman R.L., Schnur J.J., Bredley E.M., Lecocq F.R. Impairment of peripheral glucose utilization in normal subjects by prolonged bed rest. J. Lab. Clin. Med. 1970;76:221-230.

(11) Berger M., Kemmer F.W., Becker K., Herberg L., Schwenen M., Gjinauci A., Berchtold P. Effect of physical training on glucose tolerance and on glucose metabolism of skeletal muscle in anesthetized normal rats. Diabetologia 1979;16:179-184

(12) Lohmann D., Fiebold F., Heilmann J., Stenger J., Pohl A. Diminished insulin response in highly trained athletes. Metabolism 1978;27:521-524.

(13) Bjorntorp P., Berchtold P., Grimby G., Lindholm B., Sanne H., Tiblin G., Wilhelmson L. Effect of physical training on glucose tolerance, plasma insulin and lipids, and on body composition in man after myocardial infarction. Acta Med. Scand.1972;192:439-443.

(14) Bjorntorp P., Holm G., Jacobson B., Schiller-De-Jorgenge K., Lundberg P., Sjorstorm L., Smith U., Sullivan L. Physical training in human hyperplastic obesity IV. Effect on the hormonal status. Metabolism 1977;26:319-328.

(15) Galbo J., Richter E.A., Holst J.J., Christensen N.J. Diminished hormonal response to exercise in trained rats. J. Appl. Physiol. 1977; 43:953-958.

(16) Akiyoshi H., Iwamoto M., Nakaya Y. Lactate stimulates insulin secretion without blocking K+ channels in HIT-T15 inuslinoma cells. Horm. Metab. Res. 1999; 31:257-261.

(17) Hedeskov C.J. Mechanism of glucose-induced insulin secretion. Physiol. Rev.1980; 60:442-509.

(18) Vettor R., Lombardi A.M., Fabris R., Serra R., Pagano C., Macor C., Federspil G. Substrate competition and insulin action in animal models. Int. J. Obes. 2000; 24 (suppl.2):S22-S24.