Role of Insulin as a Portal Factor in Maintaining the Viability of Liver

KAZUE OZAWA, M.D., TOSHIHIKO YAMADA, M.D., ICHIO HONJO, M.D.

From the Department of Surgery, Kyoto University Faculty of Medicine, Kyoto, Japan

The subcutaneous administration of insulin did not induce a significant effect on oxidative phosphorylation of the mitochondria from normal rat liver. However, in rats subjected to ligation of a branch of portal vein, the phosphorylative activity of the mitochondria from the ligated lobe deprived of portal blood fell rapidly and at 12 hours decreased to approximately 50% of nomal liver mitochondria. After the insulin administration, the phosphorylative activity of the mitochondria from ligated lobe was rapidly stimulated within 30 minutes, reached the maximal level of normal liver mitochondria at 2 hours ($p < 0.005$) and then fell to subnormal levels. The respiratory control ratio, state 3 respiration and P/O ratio remarkably increased in parallel with an increase of phosphorylative activity. The contents of respiratory enzymes making up ATP remained unchanged. Considering the previous report that a factor, which is capable of stimulating oxidative phosphorylation of the liver mitochondria, is present in portal blood, it is suggested that insulin may play an important role in the mechanism by which the portal blood controls mitochondrial metabolism.

THE FUNCTIONAL STATUS of the liver is dependent upon portal blood supply. It is well recognized that, although it prevents the bleeding from varices of the upper gastrointestinal tract with decompressing the portal venous system, a portacaval shunt results in progressive deterioration of hepatic function. Bekoe *et al.*[1] have reported that derangement of liver mitochondria is more severe in the end-to-side shunted animals than in the side-to-side shunted animals and a major factor in initiating hepatic insufficiency following portacaval shunt. More recently, studies from our laboratory have shown, using portacaval shunted animals subjected to ligation of a branch of portal vein, that a portal factor, which stimulates the mitochondrial phosphorylative activity, is present in portal blood[9] and contributes significantly to maintain the delicate energy balance in damaged liver and the ability of the liver to regenerate.[3] It has been suggested that rapid alteration in mitochondrial metabolism in response to changes in portal blood supply is mediated by quantitative changes of the portal factor available to liver mitochondria. The role of the portal factor, however, in the control of liver mitochondria under physiological conditions remains conjectural. In this study, evidence will be presented that insulin plays an important role in the mechanism by which the portal blood controls the mechanism of liver mitochondria.

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All correspondnece to: Dr. Kazue Ozawa, the Department of Surgery, Kyoto University Faculty of Medicine, Sakyo-ku, Kyoto, Japan.

Methods

Adult male rats of the Wistar strains weighing 150–200 gm were used in this investigation. Operations were carried out on rats under ether anesthesia. The left branch of the portal vein was ligated. In this way about 60% of the hepatic tissue was deprived of its portal blood supply. Insulin (crystalline, 0.35 units/mg, Sigma) was subcutaneously injected at the dosages indicated in the text simultaneously with intragastric injection of 400 mg of glucose per 200 gm of body weight. Liver mitochondria were prepared by the method of Ozawa *et al.*[8] Oxygen consumption was measured polarographically with a rotating electrode according to the method previously described.[7] Phosphorylative activity was measured simultaneously with oxygen utilization by following the changes in pH with glass electrode. For the assay of respiratory enzymes of liver mitochondria, the spectrophotometric techniques are described elsewhere.[8] Protein

TABLE 1. *Changes of Oxidative Phosphorylation of the Mitochondria in Normal Rat Liver after Insulin Administration*

	Glutamate				Glutamate + Succinate			
	RC	State 3	P/O	PR	RC	State 3	P/O	PR
Control	4.7 ± 0.1 (8)	46.8 ± 0.9	2.3 ± 0.05	108.0 ± 5.5	5.2 ± 0.1	81.5 ± 0.9	1.8 ± 0.005	148.5 ± 8.1
Insulin	4.3 ± 0.3 (6)	58.5 ± 0.8	2.1 ± 0.1	124.3 ± 7.0	4.3 ± 0.3	85.2 ± 3.2	1.7 ± 0.1	158.9 ± 12.1

Values are means and standard errors. Values in parenthesis indicate number of animals. Oxygen consumption and phosphorylative activity were measured at 22 C at pH 7.4 in medium containing 0.3 M mannitol, 0.01 M KCl, 0.004 M $MgCl_2$, 0.01 M Tris-HCl buffer, 0.005 M potassium phosphate buffer, and 0.2 mM EDTA. Glutamate and succinate were added at a concentration of 4 mM. The respiratory control ratio was calculated from the polarographic tracings by the method of Chance[2] from the equation, RC ratio = state 3 respiration rate (in the presence of ADP)/state 4 respiration rate (after exhaustion of ADP). State 3, state 3 respiration (mμatoms/mg protein); P/O, moles of ATP formed per atom of oxygen consumed. PR, phosphorylation rate per mg protein (mμmoles ATP synthesis/mg/minute).

was determined by the method of Lowry *et al.* with crystalline bovine serum albumin as standard.[6]

Results

Table 1 shows the rates of oxidative phosphorylation of liver mitochondria of normal rats 2 hours after the administration of 2.5 unit per kg of insulin. In the respiratory control ratio, state 3 respiration, P/O ratio and phosphorylative activity, significant differences were not observed between insulin-treated and non treated rats. The findings raise the possibility that *in vivo* concentration of endogenous insulin reaching liver via portal system may be present in sufficient excess so as not to be rate limiting and to permit a maximal increase in the capacity for oxidative phosphorylation. Thus, in order to exclude the effect of endogenous insulin, a part of liver (ligated lobe) was deprived of portal blood supply with ligation of a branch of portal vein and used as a model for studying the mechanism of insulin action in more closely physiological conditions.

The phosphorylative activity of liver mitochondria rapidly fell after ligation of a branch of portal vein and at 12 hours was decreased to approximately 60 mμmoles ATP synthesized/mg protein/minute with glutamate as a substrate at 22 C, which were constantly maintained throughout the period studied (Fig. 1, a dotted line). From the results, the ligated lobe deprived of portal blood for 24 hours was used as a material.

Figure 2 shows the changes in phosphorylative activity of the mitochondria from ligated lobe 2 hours after the administration of various amounts of insulin. The phosphorylative activity of mitochondria from ligated lobe was remarkably stimulated to that of normal liver mito-

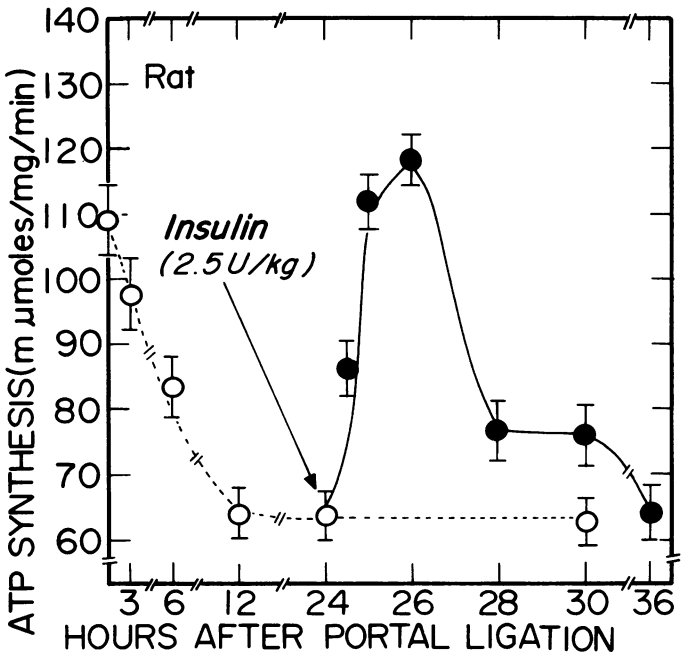

FIG. 1. Time course of changes of phosphorylative activity after ligation of a branch of portal vein (dotted line) and after insulin administration (solid line). Experimental details are given in Table 1. Each point represents the mean and standard error of values for eight or more animals.

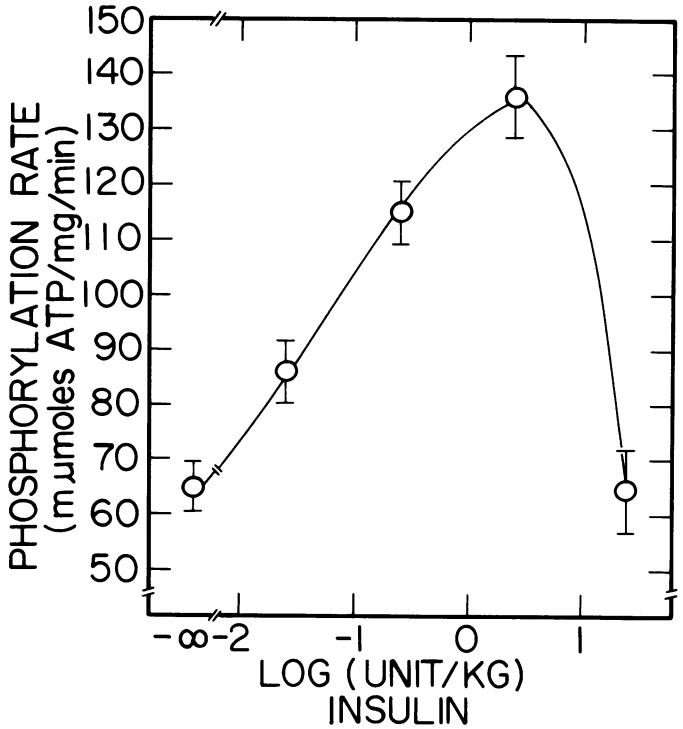

FIG. 2. Dose-response curve of insulin in phosphorylative activity of mitochondria of ligated lobe. Experimental details are given in Table 1. Each point represents the mean and standard error of values for eight or more animals.

TABLE 2. *Changes of Oxidative Phosphorylation of the Mitochondria in Ligated Lobe after Insulin Administration*

Hours after Insulin Administration	Glutamate				Glutamate + Succinate			
	RC	State 3	P/O	PR	RC	State 3	P/O	PR
Control	2.5 ± 0.1	34.3 ± 2.5	1.8 ± 0.08	62.0 ± 2.7	2.7 ± 0.1	79.7 ± 1.5	1.2 ± 0.05	98.0 ± 1.5
0.5	3.5 ± 0.2	44.5 ± 2.6	1.9 ± 0.04	87.0 ± 7.2	4.3 ± 0.02	108.0 ± 2.8	1.5 ± 0.03	164.0 ± 0.01
1	4.5 ± 0.3	51.8 ± 3.2	2.1 ± 0.1	108.4 ± 6.7	4.4 ± 0.1	117.5 ± 3.4	1.3 ± 0.04	155.0 ± 5.4
2	4.5 ± 0.2	52.0 ± 1.5	2.2 ± 0.05	114.8 ± 3.8	4.4 ± 0.2	108.9 ± 4.2	1.6 ± 0.06	175.4 ± 4.3
4	4.0 ± 0.1	42.0 ± 0.9	1.9 ± 0.08	80.1 ± 2.7	3.5 ± 0.1	96.8 ± 3.1	1.3 ± 0.05	122.7 ± 7.2
6	3.5 ± 0.2	44.2 ± 3.1	1.9 ± 0.06	83.2 ± 3.6	4.1 ± 0.2	89.5 ± 4.9	1.3 ± 0.05	116.0 ± 2.5
12	3.3 ± 0.1	31.7 ± 1.2	2.3 ± 0.04	71.8 ± 2.6	3.6 ± 0.2	80.6 ± 3.9	1.4 ± 0.02	117.0 ± 4.1

See footnotes to Table 1.

chondria with the administration of 0.25–2.5 unit per kg of insulin. The higher and lower concentrations did not result in a significant effect.

The changes of phosphorylative activity occurring in the mitochondria from ligated lobe following the administration of 2.5 unit per kg of insulin is shown in Fig. 1 (a solid line). The phosphorylative activity was rapidly stimulated within 30 minutes after the administration of insulin, reached 200% of control at 2 hours ($p < 0.005$) and then fell to subnormal level. The short period of maximal phosphorylative activity following insulin administration is an important characteristic of the physiological action. The respiratory control ratio, state 3 respiration and P/O ratio were maximally increased in parallel with an increase of phosphorylative activities with glutamate or glutamate plus succinate, as shown in Table 2. However, the contents of respiratory enzymes making up ATP remained unchanged (Table 3). On the other hand, 2 hours after the administration of glucose only the oxidative phosphorylation with glutamate as a substrate was slightly stimulated, possibly due to an increase in plasma insulin concentration occurring after glucose loading[4] (Table 4).

Discussion

It has been found in this laboratory that the interplay between a factor in portal blood and phosphorylative activity of liver mitochondria constitutes an important aspect in the excellent homeostasis for generation of energy.[3,9] The results presented herein indicate that, in an experimental *in vivo* conditions, insulin can enhance the mitochondrial phosphorylative activity depressed with portal deprivation. In addition, more recent studies showed that incubation of slices of the liver deprived of portal blood supply with insulin causes maximal enhancement of the phosphorylative activity of the mitochondria, but incubation with glucagon is without effect.[13] Although a number of factors might be involved in the mechanisms, it is clear that liver mitochondrial metabolism is, at least, regulated by an intrahepatic mechanism which is directly responsive to circulating insulin concentration. Considerable experimental evidences showed that insulin exerts multiple biological action.[15] However, there are no reports to show that regulation of mitochondrial phosphorylative activity is the fundamental physiological action of insulin. Also, at present, not enough is known regarding the regulatory action of insulin to interpret the observed responses. However, the mechanism of a beneficial effect of insulin on liver mitochondrial metabolism is a striking contrast to an increase of mitochondrial respiratory and phosphorylative activities following the administration of thyroid hormone. Tata[16] has concluded that a primary physiological effect of thyroid hormone is to increase biosynthetic processes controlling enzymes or structural elements of respiratory activity. An enhancement of oxidative and phosphorylative activities following the insulin administration appears to be due to different mechanism, since the contents of respiratory enzymes remain unchanged. The stimulated effect of insulin on mitochondrial metabolism appears to be localized at any single rate-limiting step of either the electron transport or phosphorylation

TABLE 3. *Changes of the Contents of Respiratory Enzymes after Insulin Administration*

	a(+a_3)	b	c + c_1	fp	PN
	10^{-10} moles/mg protein				
Normal Liver (6)	2.22 ± 0.08	0.92 ± 0.08	2.50 ± 0.06	7.2 ± 0.2	51.9 ± 1.6
Control (24 hours-ligated lobe) (8)	1.91 ± 0.09	0.82 ± 0.09	2.24 ± 0.06	8.3 ± 0.3	61.6 ± 2.6
Insulin-treated (24 hours-ligated lobe) (8)	2.13 ± 0.15	1.17 ± 0.05	3.12 ± 0.05	8.2 ± 0.4	55.5 ± 3.0

Insulin of 2.5/kg was administrated 24 hours after ligation of a branch of portal vein. The mitochondria were prepared from the ligated lobe 2 hours after insulin administration.

TABLE 4. *Changes of Oxidative Phosphorylation of the Mitochondria in Ligated Lobe 2 Hours after Administration of Glucose Only or Glucose Plus Insulin*

	Glutamate				Glutamate + Succinate			
	RC	State 3	P/O	PR	RC	State 3	P/O	PR
Control	2.5 ± 0.1	34.3 ± 2.5	1.8 ± 0.08	62.0 ± 2.7	2.7 ± 0.1	79.7 ± 1.5	1.2 ± 0.05	98.0 ± 1.5
Glucose	4.0 ± 0.3	39.5 ± 1.0	2.1 ± 0.07	83.0 ± 4.1	3.3 ± 0.1	82.7 ± 2.7	1.2 ± 0.02	101.6 ± 2.3
Glucose plus Insulin	4.5 ± 0.2	52.0 ± 1.5	2.2 ± 0.05	114.8 ± 3.8	4.4 ± 0.2	108.9 ± 4.2	1.6 ± 0.06	175.4 ± 4.3

See footnotes to Table 1.

system. The increase in the respiratory control ratio and P/O ratio suggests that a regulatory device such as conformational change in respiratory enzyme system participates in these mechanisms.

It is well recognized that portacaval shunting results in progressive deterioration of hepatic function. It is tempting to speculate that in the patients with decrease of portal flow rate (advanced liver cirrhosis) or diversion of the portal supply from the liver one of the most important causes for liver insufficiency is a reduction of insulin level per hepatic cell. This implies a basic defect in mitochondrial energy production which results in hepatic insufficiency, because hepatic cell metabolism is strongly dependent on the continuous supply of high energy phosphate mainly produced with the mitochondria. On the other hand, considering that redirecting the pancreatic venous drainage to the systemic circulation prevents the destruction of a major fraction of the secreated insulin and more insulin is available for the peripheral tissues, LeVeen *et al.*[5] have suggested that redirecting the pancreatic venous blood from the portal circulation to the systemic circulation dramatically alleviates the diabetes. However, since reduction of insulin concentration available to hepatic cell is not adequate to maintain functional integrity of hepatic cells, more studies appear to be required to apply such operation clinically.

Current investigations in this laboratory are concerned with elucidating metabolism of liver mitochondria in various liver diseases.[10,11] More recently, it has been found that, in the patients with liver cancer localized to one lobe, the phosphorylative activity of the mitochondria from tumor-bearing lobe is inhibited, while that from the lobe without cancer is enhanced.[12] Also, it has been found that in the mitochondria from regenerating liver of rat treated by chloramphenicol the oxidative and phosphorylative activities per unit of respiratory carriers are remarkably increased inversely with decreasing contents of cytochrome a($+a_3$), b and c_1.[14] Those indicate the presence of an excellent homeostatic mechanism in maintaining the delicate energy balance in liver. Insulin may play an important role in this mechanism. Further studies are under way.

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