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EFFECTS OF PALMITIC ACID ON THE BINDING OF INSULIN-^{131}I TO THE RAT LIVER MITOCHONDRIA

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ABSTRACT

Effects of palmitic acid, inhibitors, uncouplers, and reduced and oxidized glutathione on the binding of insulin-^{131}I and ^{131}I to the isolated fresh or aged mitochondria were studied.

The binding of insulin-^{131}I to the fresh mitochondria was unexpectedly increased to a greater degree with the exogenous addition of palmitic acid, glutathione, acetaldehyde and 2, 4-dinitrophenol to the medium. The fact indicated that the intactness of mitochondria was not prerequisite for the binding of insulin-^{131}I to the isolated mitochondria.

The binding of insulin-^{131}I was compared to the fresh mitochondria and the aged mitochondria. Insulin-^{131}I bouud to the aged mitochondria in a lesser degree. It is suggested that the binding of insulin-^{131}I to the mitochondria might be related to the mitochondrial functional and conformational state.

The binding of ^{131}I to the mitochondria was apparently differrnt from that of insulin-^{131}I. And the binding of ^{131}I was not altered by these uncouplers except in the case of acetaldehyde and ethyl alcohol. This implies that the process of binding of insulin-^{131}I is different from that of ^{131}I. In the case of ^{131}I, it may have some connections with its ion transport mechanism.

INTRODUCTION

Insulin is reported to induce the multiple effects to the biological function. Levine[1] postulated that the action of insulin can be reduced to the one effect that stands at the apex of the hierarchial pyramid, and which is fundamental to each of the others. However, this concept is not yet experimentally supported. Recently, it was reported that in the lymph vessels as well as in the urine, relatively large amounts of immunoreactive insulin are detected and that insulin can penetrate the cell membrane[2]. It is highly probable that insulin is capable of traversing the cell membrane and interacting with the cell constitution such as mitochondria. Lee and Weisman[3] found that insulin-^{131}I is associated with the rat liver mitochondria after injection, but the possibility is not neglected that the insulin binds to the cell particles during homogenization of the liver. The binding of insulin-^{131}I to the isolated rat liver mitochondria was studied in detail[3]. But the physiological significance of the

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binding of exogenous added insulin-^{131}I to the mitochondria was not clear. It was found that uncoupled mitochondrial AMP/O ratio due to palmitic acid or aging recovered to normal level with the addition of insulin *in vitro*[4]. These facts suggested that insulin bound to the mitochondrial membrane resulting in the amelioration of mitochondrial function and induced some physiological significant effects.

In this reports, it was intended to observe the changes of binding capacity of insulin-^{131}I to the isolated rat liver mitochondria, and inquire into the physiological significance of its binding effects.

MATERIALS AND METHODS

Male albino rats (wistar strain) weighing from 200 to 210 grams, were used in all experiments and were fed with the stock diet, oriental pellets from the Oriental company, properly balanced with carbohydrate, protein, fat, minerals and vitamines and they had free access to the water.

The mitochondria were prepared according to Chance and Hagihara[5] by the procedure listed in Table 1. The prepared mitochondria were used as soon as possible as fresh mitochondria.

TABLE 1. The Preparation of Mitochondrial Suspension

Rat liver 3 g+27 ml of the mixture (0.21 M Mannitol, 0.27 M Sucrose, 0.1 mM EDTA)
↓
Homogenized
↓
Add 30 ml of the mixture
↓
Centrifuge 700×g (10 minutes)
↓
Centrifuge supernatant 8,000×g (10 minutes)
↓
Sediment+30 ml of the mixture
↓
Centrifuge 8,000×g (10 minutes)
↓
Sediment+Approriate volume of the mixture

Parts of fresh mitochondrial suspension were kept at 4°C for 24 hours, and they are called aged mitochondrial suspension.

Respiratory control index (R.C.I.) and AMP/O ratio were measured to test the physiological condition of mitochondria. For the measurement of AMP/O and R.C.I., the reaction system was 0.3 M in mannitol, 10 mM in KCl, 10 mM in phosphate, pH 7.4, 2.5 mM in $MgCl_2$, 0.25 mM in EDTA, 2 mM in succinate and 0.2 mM in AMP. And the concentration of mitochondrial protein was 2 mg/ml and total volume of the reaction system was five ml. According to Chance and Williams[6], the rate of oxygen uptake was measured by polarographic oxygen electrode (Beckman's oxygen sensor). The R.C.I. and AMP/O

ratio were calculated by the method of Chance and Williams[6].

For the experiments of the binding of insulin-^{131}I to the mitochondria, mitochondria (10 mg protein) were incubated for 30 min. at 20°C in two ml of the above described reaction system with 4 μC of insulin-^{131}I (from the *Dinabot* Radio Isotope Lab., LTD., 174 mC/mg) or 4 μC ^{131}I (from the Japan Isotope Society) and with or without addition of 1 mM palmitate, 2.5 mM glutathione, 60 mM ethanol, 60 mM acetaldehyde and 50 μM 2, 4-DNP. After incubation, each tubes were centrifuged with 8,000 g for 10 min. To each sediments, two ml of the reaction mixture was added and washed with homogenizing and centrifuged with 8,000 g. This procedure was repeated five times until the supernatant radioactivity was become negligible. The sediments were solubilized in two ml of reaction mixture at the final step. All the supernatant was collected. And the radioactivity was measured with a gamma counter. Results were expressed as per cent radioactivity of the total sediments to that of the total radioactivity (sum of radioactivity in sediment and supernatant).

RESULTS

Freshly prepared mitochondrial suspension showed good R.C.I. and AMP/O. But the aged mitochondrial suspension showed not so good R.C.I. and AMP/O which suggested that the aged mitochondrial oxidative phosphorylation mechanism was almost uncoupled as listed in Table 2.

TABLE 2. AMP/O and Respiratory Control of Fresh or Aged Mitochondrial Suspension

	Fresh mitochondria			Aged mitochondria		
AMP/O	1.4	1.3	1.4	0	0	0
R.C.I.	6.1	6.3	5.9	1.1	1.5	1.2

Liver mitochondria were prepared from three rats. Freshly prepared mitochondrial suspension was named as fresh mitochondria in this case. One part of fresh mitochondrial suspension was kept at 4°C for 24 hours. This aged sample was named as aged mitochondria.

Binding of insulin-^{131}I to the aged mitochondria was 0.45±0.15% of total activity and this ratio was not changed with the addition of ethyl alcohol as shown in Fig. 1. But this binding ratio to the aged mitochondria was significantly elevated by the addition of palmitic acid, reduced or oxidized glutathione, acetaldehyde, and 2, 4-DNP. These values were 2.56±0.92, 2.05±0.64, 2.09±0.73, 2.96±0.82, 1.12±0.42% of total activity respectively. The binding of ^{131}I to the aged mitochondria was 0.55±0.17% of total activity. This ratio was elevated up to 1.3±0.69% only with the addition of acetaldehyde (Fig. 1).

Binding of insulin-^{131}I to the fresh mitochondria was apparently different from the data obtained from the aged mitochondria as shown in Fig. 2. Bind-

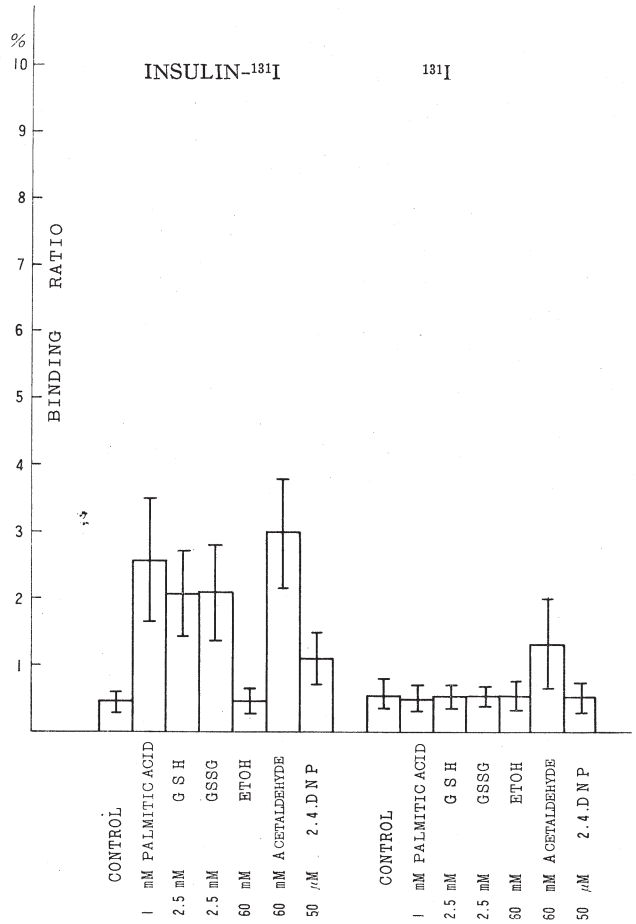

FIG. 1. The binding of insulin-^{131}I and ^{131}I to the aged mitochondria.

4 μc insulin-^{131}I or 4 μc ^{131}I and aged mitochondria containing 10 mg protein were incubated in the test tubes at 20°C for 30 min with or without the listed in the figure. Total volume of the reaction system was 2 ml, which contained 0.3 M in mannitol, 10 mM in KCl, 10 mM in phosphate, pH 7.4, 2.5 mM in $MgCl_2$, 0.25 mM in EDTA, 2 mM in succinate and 0.2 mM AMP. And the procedure was as described in the text. The percent of the radioactivity of the sediment to the total radioactivity (sum of the radioactivity in the sediment and the supernatant) was expressed as the binding ratio. Each bar represents mean binding ratio±S.E. of four test tubes in each case. The binding ratio of the test tube without addition was expressed as the control value.

ing of insulin-^{131}I to the fresh mitochondria was 0.18±0.61%, which was higher than the binding ratio obtained with the aged mitochondria. This ratio was significantly elevated with the addition of palmitic acid, reduced or oxidized glutathione, acetaldehyde and 2, 4-DNP, but lowered with the addition of ethyl-alcohol. These binding ratios of insulin-^{131}I to the fresh mitochondria were

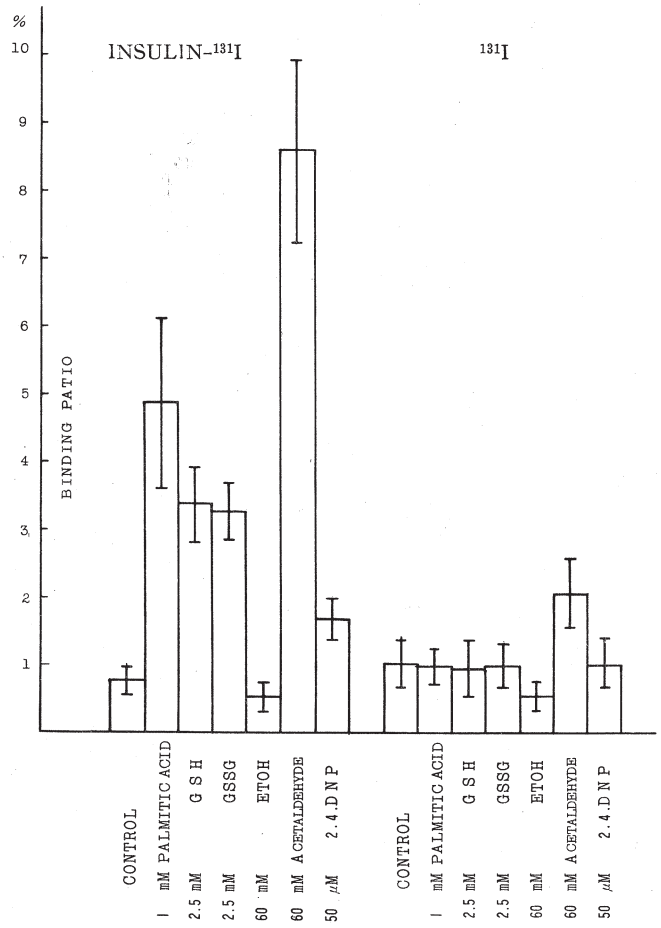

FIG. 2. The binding of insulin-^{131}I and ^{131}I to the fresh mitochondria. The experimental conditions were the same as in Fig. 1 except using the fresh mitochondria in place of the aged mitochondria.

4.9±1.25, 3.38±0.54, 3.26±0.44, 1.72±0.31, 8.61±1.35% of total activities, respectively. The binding ratio by mitochondria treated with ethyl alcohol was 0.56 ±0.21%.

Binding ratio of ^{131}I to the fresh mitochondria was 1.1±0.74%, and this ratio was higher than that of ^{131}I to the aged mitochondria. The binding ratio of ^{131}I to the fresh mitochondria was unchanged with the addition of palmitic acid, glutathione, 2, 4-DNP, but elevated with the addition of acetaldehyde and lowered with the addition of ethyl alcohol. The binding ratio of ^{131}I to the fresh mitochondria with addition of ethyl alcohol was 0.55±0.19%, and the ratio with acetaldehyde was 2.05±0.49% (Fig. 2). But the results from Fig. 1 and Fig. 2 as for bindings of ^{131}I showed that the tendency between fresh and aged

mitochondria to the various exogenous additions was almost same.

DISCUSSION

The binding of insulin-^{131}I to the mitochondria was unexpectedly increased by the addition of palmitic acid, acetaldehyde and 2, 4-DNP. Because it was found that acetaldehyde was one of the inhibitors[7], all of these additions gave uncoupling to the mitochondrial oxidative phosphorylation. Therefore, the intactness of mitochondria is suspected to be not prerequisite to the binding of insulin-^{131}I to the mitochondria. These findings suggest that insulin binds to the mitochondria through the complicated process, which has the cross connection with the structure and function of the mitochondria. The addition of reduced or oxidized glutathione also induced an increased binding of insulin-^{131}I to the mitochondria. Lehninger[8] proposed that insulin interacts with mitochondrial membrane through disulfide-sulphydryl interchange reaction. The addition of glutathione is suspected to effect the concomitant conformational changes of mitochondrial membrane which are one of the factors to influence the binding of insulin to the mitochondria.

The aged mitochondria apparently showed less affinity to bind with insulin-^{131}I compared with that of fresh mitochondria, but the tendency was almost same. And the aged mitochondria is shown to have the decreased level of phospholipid in membrane with increased activity of phospholipase A[9]. These facts are suggesting that the binding of insulin has some cross connections with mitochondrial structure and functions.

On the contrary to the data of insulin-^{131}I, the binding of ^{131}I to the mitochondrial membrane was not altered by palmitic acid, 2, 4-dinitrophenol, reduced and oxidized glutathione. This indicates that the processes of the binding of ^{131}I are different from that of insulin-^{131}I. As for binding of ^{131}I, it might be discussed as relation to the ion transport.

It is interesting to find that acetaldehyde induced an increase of binding of ^{131}I and insulin-^{131}I to the mitchondria. The effects of acetaldehyde to the mitochondrial function are now under study.

Christophersen[10] reported that ethyl alcohol changes the permeability of mitochondria for the β-hydroxybutyrate. In our experimental condition, ethyl alcohol reduced the binding of insulin-^{131}I and ^{131}I to the fresh mitochondria. The mechanism of these alcohol effect are not clarified but might be partially due to the changes of lipoprotein fraction in a membrane with the increased permeability, resulting in the decreased binding of ^{131}I or insulin-^{131}I to the mitochondria.

It has been found that the uncoupled mitochondrial oxidative phosphorylation due to the addition of palmitic acid recovered to normal level by the addition of insulin[4]. It is also rational to suppose that insulin has chances to

ameliorate the uncoupled mitochondroal function through the increased binding of insulin-^{131}I.

Whether the binding of insulin to the isolated mitochondrial membrane has some physiological significances even *in vivo* or not is the important problem which is not yet solved. Recently, Ryser[2] reported that insulin can penetrate the cell membrane. There are some posibilities that insulin in the plasma can pass through the membrane of cell and interact with the cell constituent, such as mitochondria, resulting in the metabolic regulation of the mitochondrial function.

SUMMARY

There are many data showing the binding of insulin-^{131}I to the mitochondria, but physiological significance of the binding of insulin-^{131}I to the mitochondria is not clearly elucidated. It is reported in this paper that the binding of insulin-^{131}I to the fresh mitochondria was higher than to the aged mitochondria and the binding ratio was apparently influenced by various condition.

The binding of ^{131}I to the mitochondria was not so changed by the various condition which influenced the mitochondrial function.

These data showed that the conformational and biochemical alteration of mitochondrial membrance had cross connection with the binding of insulin-^{131}I to the mitochondria.

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