INSULIN RECEPTOR DEFECT IN INSULIN RESISTANCE: STUDIES IN THE OBESE-HYPERGLYCEMIC MOUSE

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SUMMARY. To evaluate the possible role of the insulin receptor in the pathophysiology of disease, the interaction of insulin with plasma membranes of liver has been studied in the obese hyperglycemic mouse and their thin litter mates. The obese hyperglycemic syndrome is characterized by marked resistance to both endogenous and exogenous insulin. Under identical conditions of purification and incubation, plasma membranes of the obese mouse bind only 16-35% as much ^{125}I-insulin as membranes of the thin mouse. This is in contrast to other characteristics of the membrane which are similar to these two animals.

The first step in insulin action appears to be interaction with receptor sites on the plasma membranes of target cells. This reaction has been studied directly by binding of radioactively labeled insulin to a variety of receptor preparations including purified plasma membranes of rat liver (1,2), isolated fat cells (3,4), and human lymphocytes (5). Bennett and Cuatrecasus (6) have also examined insulin binding by isolated fat cells in rats that had been treated with prednisone or streptozotocin and in rats that had been starved and found no alteration in this reaction.

The obese-hyperglycemic syndrome in mice is a recessively inherited trait in which there is marked obesity and resistance to both endogenous and exogenous insulin (7). The extreme insulin resistance is demonstrated by the convulsive dose for exogenous insulin which in these mice is above 600 U per kilogram body weight (8). The data presented in this preliminary report suggests that in these animals there is a

decrease in the insulin receptors on the liver plasma membrane, and this may be important in the resistance which they show.

METHODS

C57Bl/6J mice were obtained from Jackson Laboratories, Bar Harbor, Maine and fed ad libitum on Purina rat chow. Purified plasma membranes were prepared from livers of the obese-hyperglycemic animals (ob/ob) and their thin litter mates using the method of Neville (9). ^{125}I-insulin was prepared as described in detail elsewhere (2). Binding studies were conducted at 30° for 30 minutes in a Krebs-Ringer phosphate buffer. The membrane bound-hormone was separated by centrifugation in a Beckman microfuge, washed, and counted as previously described (1). All binding data are the mean of triplicate samples and are corrected for "non-specific binding" by subtracting from the total binding that amount which is bound in the presence of 10^{-6} M native insulin.

Adenylate cyclase activity was measured by the method of Krishna, et al (10) in a medium that contained 3.3 mM ATP-α-^{32}P, 5 mM $MgCl_2$, 1 mM EDTA, 25 mM Tris-HCl, pH 7.5, and an ATP-regenerating system consisting of 20 mM phosphocreatine and creatine phosphokinase 1 mg/ml. Incubations were conducted at 30° for 10 min and terminated by boiling for 3 min. 5'-nucleotidase assays were carried out at 37° as previously described (11) with the addition of a TCA deproteinization step before adding the molybdate reagent. Protein was measured by the Lowry procedure (12).

RESULTS

At 30°, ^{125}I-insulin rapidly bound to purified liver plasma membranes of both the obese-hyperglycemic mice and their thin litter mates (Fig. 1). Maximum binding occurred after 30 minutes of incubation and slowly decreased thereafter. This fall off in binding has also been observed in studies with rat liver membranes and fat cells (4) and appears to be due to some alteration of the insulin receptor

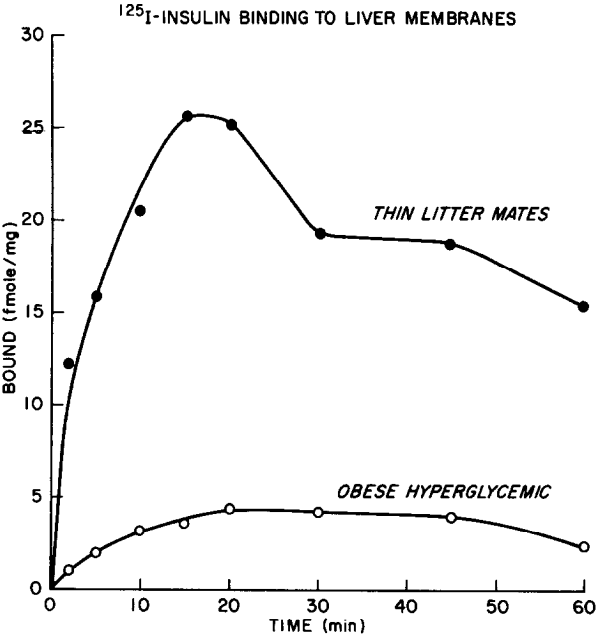

Fig. 1. Time course of binding of ^{125}I-insulin to liver membranes of obese hyperglycemic mice and their thin litter mates. The insulin concentration was 5×10^{-11} M. The membrane protein concentrations were 0.6 mg/ml and 0.5 mg/ml for the obese and thin mice, respectively.

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or degradation of insulin (2).

Under identical conditions of incubation, maximum binding by the purified plasma membranes of obese-hyperglycemic mice was only 16-35% of that obtained with identically prepared membranes of thin litter mates (Table I). The specifically bound insulin is readily displaced by unlabeled insulin at concentrations found in portal blood (Fig. 2). Scatchard analysis of the data suggests that like the receptor in purified membranes of rat liver (13), the population of receptors is not homogenous but may be represented by two or more orders of sites. The difference in binding appears to be due primarily to a decrease in the number of receptor sites in the membrane of the obese mouse liver.

Possible causes of a spurious reduction in binding were also

TABLE I

INSULIN BINDING IN LIVER MEMBRANES OF THIN AND OBESE-HYPERGLYCEMIC MICE

Experiment	Phenotype	^{125}I-insulin Bound
		(fmole/mg protein)
I	Thin	200 ± 8
	Obese	35 ± 3
	Obese	70 ± 6
II	Thin	368 ± 18
	Obese	103 ± 16

Table I. The membrane protein concentrations was 0.33 ng/ml for all experiments. The concentration of ^{125}I-insulin used was 2×10^{-10} and 5×10^{-10} M in experiments I and II, respectively. Data are expressed as Mean ± S.E.M. for triplicate samples.

TABLE II

EFFECT OF INSULIN INJECTION AND MIXING ON BINDING

Phenotype	Treatment	^{125}I-insulin Bound
		(fmole/mg protein)
Thin	None	205 ± 10
Thin	Insulinized	183 ± 3
Obese	None	54 ± 5
Thin and Obese	Equal Mixture	130 ± 3

Table II. The membrane protein concentration was 0.33 ng/ml and the ^{125}I-insulin concentration was 2×10^{-10} for all experiments. The thin insulized animals received 1 mg of insulin subcutaneously 90 min. prior to sacrifice (see text). Data are all expressed as Mean ± S.E.M. for triplicate samples.

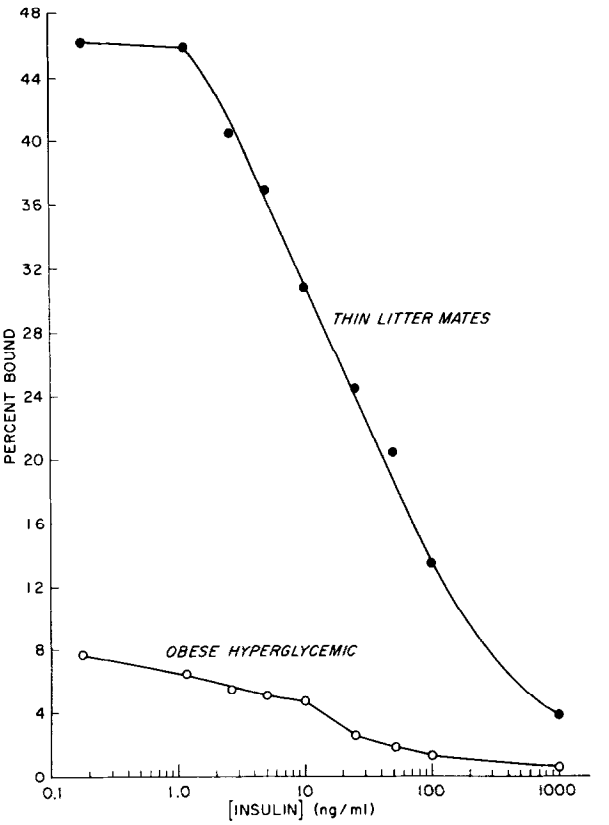

Fig. 2. Displacement of ^{125}I-insulin by native insulin. The membrane protein concentration was 0.5 mg/ml for both preparations, the ^{125}I-insulin concentration was 3×10^{-11}M. Each point is the mean of duplicate determinations.

considered. Insulin binding observed with a mixture of membranes of the obese and thin mouse failed to reveal any inhibitor of binding (Table II). To evaluate the effect of high circulating insulin concentration, thin mice were injected with 1 mg of insulin subcutaneously and sacrificed 90 minutes later while having hypoglycemic convulsions. Membranes prepared from these animals showed little or no reduction in insulin binding (Table II).

In contrast to the difference in insulin binding capacity of the membrane preparations, other studies revealed little difference.

TABLE III

ENZYME ACTIVITIES IN MOUSE LIVER MEMBRANES

ENZYME	ACTIVITY IN LIVER MEMBRANE	
	Obese-Hyperglycemic Mouse	Thin Litter Mate
	(μmoles/hr/mg protein)	
5'-Nucleotidase (3)	8.5 ± 1.6	7.7 ± 0.8
	(nmoles/10 min/mg protein)	
Adenylate Cyclase		
Addition: None (6)	0.55 ± .29	0.44 ± .12
NaF 10mM (3)	0.77 ± .05	0.78 ± .16
NaF 20mM (3)	0.98 ± .17	1.06 ± .41
Glucagon (3)	1.48 ± .09	1.54 ± .12

Table III. Enzymes were assayed as described in Methods. The glucagon concentration used in the adenylate cyclase assay was 20 ug/ml. Data are expressed as the mean ± S.D. for the number of samples in parenthesis.

The activities of the membrane bound enzymes, 5'-nucleotidase and adenylate cyclase, were similar in membrane preparations of both thin and obese mice (Table III). Both glucagon and fluoride ion stimulate the adenylate cyclase activity and the stimulated levels are also comparable in the two animals. The values of 5'-nucleotidase are similar to those reported by Beneditti and Emmelot (14) for purified membranes of mouse liver and are three to four-fold lower than observed in purified plasma membranes of rat liver. The value of adenylate cyclase are also slightly lower than observed in preparations of rat liver membrane (15).

DISCUSSION

The data presented in this study represent the first direct measurement of the insulin-receptor interaction in the obese-hyperglycemic mouse. Our findings suggest that in this syndrome there is a very significant decrease in the number of receptors, and that this may, at least in part, account for the insulin resistance observed in these animals. Although the data are expressed for the purified membranes only, we have measured the cell size and find that there is decreased insulin binding both per cell and per unit surface area (16). The similar levels of 5'-nucleotidase and adenylate cyclase also suggest that if there is some stretching of the membrane in the cells of the livers of the obese animals, other activities of the membrane remain normal.

From these preliminary studies, it is not clear whether this alteration in receptors is the primary inherited defect in this strain of mice or whether it may be secondary to obesity, chronic hyperinsulinism or some other factor. Further studies are in progress to fully characterize these membranes, to try to answer the question of insulin binding per cell by direct study, and to find out what factors contol the level of receptors in these animals. Studies of this and similar animal models may yield valuable information regarding the role of the insulin receptor in pathologic states in man.

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