INSULIN-RECEPTOR INTERACTIONS IN LIVER CELL MEMBRANES

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SUMMARY. The specific binding of ^{125}I-insulin to liver cell membranes is a saturable process with respect to insulin. Binding is displaced by low concentrations of native insulin but not by biologically inactive insulin derivatives or by other peptide hormones. The rate constants of association (3.5×10^6 $mole^{-1}$ sec^{-1}) and of dissociation (2.7×10^{-4} sec^{-1}) of the insulin-membrane complex can be determined independently. The dissociation constant of the complex, determined from the rate constants and from equilibrium data, is about 7×10^{-11} M. Complex formation does not result in degradation of the insulin molecule. The binding interaction is a dissociable process involving a homogeneous membrane structure which is almost certainly the biologically significant receptor. The kinetic properties, and the effects of enzymic perturbations of the membrane, suggest that the insulin receptors of liver and of adipose tissue cells may be very similar structures.

Large agarose beads containing covalently bound insulin possess insulin-like biological activity when tested on isolated adipose tissue cells, suggesting that the receptors for this hormone are located in superficial regions of the cell (1). Sensitive techniques have been developed which permit direct and accurate measurements of specific and biologically significant insulin-receptor interactions in intact, metabolically responsive fat cells (2). The interaction is a simple, homogeneous and dissociable process which can be characterized by standard kinetic expressions.

The insulin receptor of fat cells appears to be located exclusively in the plasma membrane of these cells (2). The insulin-binding activity of intact cells is recovered quantitatively in the membrane fraction (2). A number of kinetic properties, and the effect of chemical and enzymic perturbations, are identical in the intact fat cell and in the membrane fraction (3). Thus there is reasonable assurance that study of the binding of insulin to fat cell membranes by these procedures reflects biologically specific receptor processes.

At present the interaction of insulin with receptors in liver cells can be studied only by measuring the specific binding of labeled hormone to broken-cell preparations. The properties of this binding cannot be compared directly with the properties of an intact system since it is not yet possible to measure accurately insulin binding properties in a simple, biologically responsive system from liver cells. It is hence difficult to ascertain that binding studies in liver cell fragments measure specific receptor interactions. This report describes the basic properties of the interaction between insulin and isolated liver membrane preparations. The nature of the interaction itself, and the remarkable similarities between the properties of this interaction and those observed in fat cell membranes, indicate that the biologically important receptor of liver cells is being studied.

METHODS. The liver membranes (from Sprague-Dawley rats) were prepared (4) from homogenates by differential centrifugation in 0.25 M sucrose solutions; they were separated from the major nuclear and mitochondrial elements. Protein was determined by the method of Lowry et al. (5).

^{125}I-Insulin was prepared and purified as described in detail elsewhere (2). This ^{125}I-insulin is biologically indistinguishable from native insulin (2). The assay used to measure specific binding of ^{125}I-insulin to membranes was similar to that described for measuring binding to intact fat cells (2) and fat cell membranes (3). Membranes were incubated with ^{125}I-insulin (10^{-10} M to 10^{-12} M) to equilibrium, then filtered and washed on EGWP Millipore filters. Every experimental point (performed in triplicate) is corrected for nonspecific binding of insulin by performing identical incubations in which large amounts (20 to 60 μg per ml) of native insulin are added before the iodoinsulin (2).

RESULTS. Specific binding of insulin to liver cell membranes is detected at very low concentrations (10^{-11} M) of the hormone (Figure 1). The insulin-binding sites in the membrane are saturated at 3×10^{-10} M ^{125}I-insulin. The maximal binding capacity of these membrane preparations is about 0.1 pmole of insulin per mg of membrane protein.

TABLE I. Displacement of ^{125}I-Insulin-Binding to Liver Membranes by Peptide Hormones

Liver membranes (71 μg of protein) were incubated for 30 minutes at 24° in 0.2 ml KRB buffer, 1% albumin, a peptide hormone, and 1.7×10^{-11} M ^{125}I-insulin.

Addition	Specific binding of ^{125}I-insulin
	nmoles x 10^{-6}/mg protein
No additions	4.2 ± 0.1*
Native insulin, 2 mμg per ml	3.0 ± 0.1
8 mμg per ml	2.1 ± 0.1
0.4 μg per ml	0.1 ± 0.1
Proinsulin, 0.2 μg per ml	3.4 ± 0.2
10 μg per ml	0.9 ± 0.1
Desoctapeptide insulin, 5 μg per ml	4.0 ± 0.2
Glucagon, 50 μg per ml	4.3 ± 0.1
Growth Hormone, 50 μg per ml	4.2 ± 0.1

*± standard error of the mean (three observations).

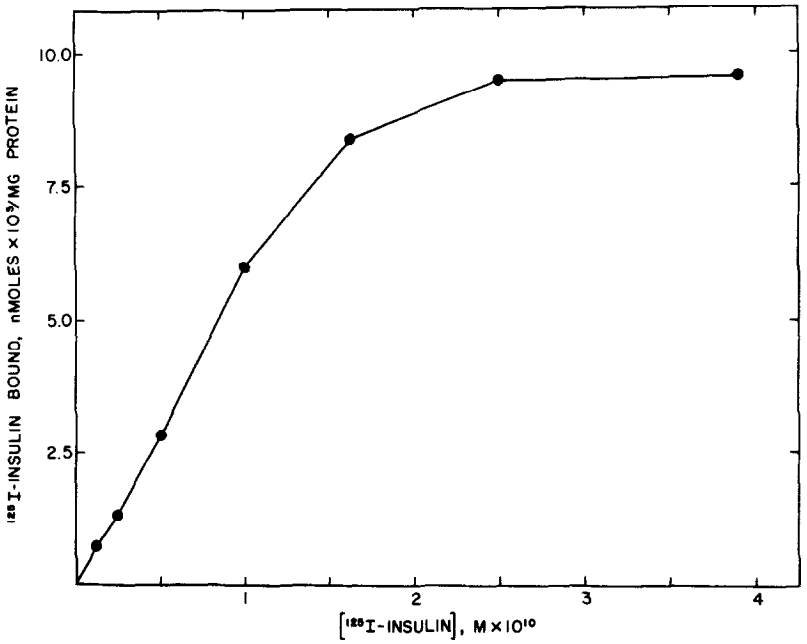

Figure 1 - Dependence of specific binding of ^{125}I-insulin to liver membranes on the concentration of insulin. Membranes (50 μg of protein) were incubated for 40 minutes at 24° in 0.2 ml of Krebs-Ringer-bicarbonate (KRB) buffer, 1% (w/v) albumin, and the indicated amount of ^{125}I-insulin. Three ml of ice-cold KRB buffer, 0.1% albumin, were added to each tube, and the suspension was rapidly filtered on EGWP Millipore filters and washed with an additional 10 ml of ice-cold buffer. Values are corrected for nonspecific adsorption of insulin, as described in the text.

The specific binding of ^{125}I-insulin to liver membranes is readily displaced by low concentrations of native insulin but not by high concentrations of glucagon or growth hormone (Table I), or by concentrations of albumin as high as 5% (w/v). The biologically inactive, reduced or oxidized chains of insulin, alone or in combination, do not affect the binding of ^{125}I-insulin (4 x 10^{-11} M) at concentrations as high as 0.2 mg per ml. Desoctapeptide insulin (devoid of biological activity) does not compete for insulin binding (Table I). Proinsulin displaces ^{125}I-insulin from the membrane (Table I) but the displacement curve indicates that its affinity is 16 times less than that of native insulin. Insulin antiserum readily dissociates the insulin-membrane complex.

The specific binding of ^{125}I-insulin to liver cell membranes is a time-dependent process which obeys second-order kinetics. The bimolecular nature of this interaction is confirmed by kinetic analyses similar to those used in the studies of fat cells (2). Dissociation of the insulin-membrane complex follows first-order kinetics (Figure 2). The rate constants are summarized in Table II. The dissociation constant calculated from the ratio of the rate constants (7.7 x 10^{-11} M) is nearly the same as that (6.7 x 10^{-11} M) calculated from the equilibrium data presented in Figure 1. These values are similar to those of the insulin-fat cell complex, which have been reported (2) as 5 x 10^{-11} M (from rate constants) and 8 x 10^{-11} M (at equilibrium).

TABLE II. Kinetic Parameters of Insulin-Receptor Interaction in Liver Membranes (at 24°)

Rate of association	3.5 ($\pm$ 0.4) x 10^{6} $mole^{-1}$ sec^{-1}
Rate of dissociation	2.7 ($\pm$ 0.2) x 10^{-4} sec^{-1}
Dissociation constant, k_{-1}/k_1	7.7 x 10^{-11} M
At equilibrium	6.7 ($\pm$ 0.8) x 10^{-11} M

No appreciable destruction of medium ^{125}I-insulin occurs during the conditions of incubation. A suspension containing 0.5 mg of membrane protein per ml

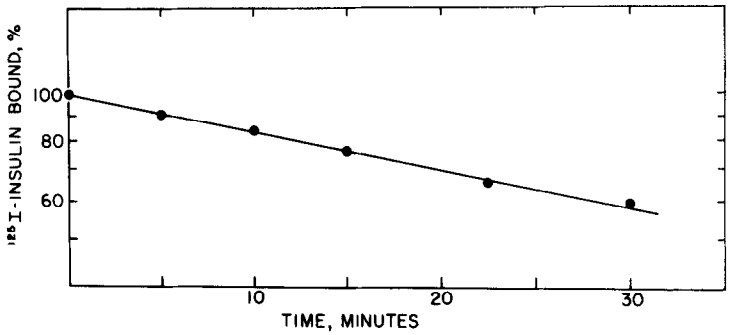

Figure 2 - Semi-log plot of the dissociation of ^{125}I-insulin bound to liver membranes as a function of time at 24°. Membranes (0.1 mg of protein) were incubated for 40 minutes at 24° in 0.2 ml of KRB buffer, 1% albumin, and 3.5 x 10^{-10} M ^{125}I-insulin. Three ml of KRB buffer, 1% albumin, (at 24°) and native insulin (10 μg per ml) were added. At the indicated times the suspensions were rapidly filtered and washed on Millipore filters.

of Krebs-Ringer-bicarbonate (KRB) buffer, 1% albumin, and 2.2 x 10^{-10} M ^{125}I-insulin was incubated for 40 minutes at 24°. The ^{125}I-insulin recovered after filtration on Millipore filters was 95% as effective as native ^{125}I-insulin when tested for binding to fresh membranes. These results are in contrast to

TABLE III. Properties of ^{125}I-Insulin Eluted from Insulin-Liver Membrane Complex

Twelve ml of KRB buffer, 1% albumin, containing 6 mg of liver membrane protein, were incubated for 20 minutes at 24° with 3.0 x 10^{-10} M ^{125}I-insulin. The suspension was centrifuged at 22,000 x g for 20 minutes. The membranes were resuspended in 12 ml of the same buffer (4°) and centrifuged again. ^{125}I-insulin was eluted from the pellet by suspending in 0.8 ml of 0.2 N HCl containing 10% albumin and incubating for 30 minutes at 24°. The suspension was centrifuged, and the supernatant was neutralized and tested by physical methods (2) and binding.

Properties	^{125}I-Insulin	
	Not used	Eluted from membranes
% Precipitable by 8% TCA	97	98
% Adsorption to talc	96	96
% Adsorption to silica	97	98
Specific binding to membranes*		
1.1 x 10^{-11} M ^{125}I-insulin	3.6 x 10^{-6}	3.8 x 10^{-6}
3.2 x 10^{-11} M ^{125}I-insulin	1.1 x 10^{-5}	1.2 x 10^{-5}

*Nmoles of ^{125}I-insulin per mg of membrane protein.

the marked ability of liver membranes to inactivate glucagon (4,6). The ^{125}I-insulin tightly bound to the liver membranes can be eluted with acid. The eluted insulin is similar to native ^{125}I-insulin by several physical criteria and by its ability to bind to fresh membranes (Table III). The binding process is not associated with degradation of insulin.

The specific binding of ^{125}I-insulin to liver cell membranes is enhanced by digesting the membranes with phospholipase C or phospholipase A, and by increasing the ionic strength of the medium (Table IV). These rather unique properties are very similar to those of the insulin receptor of intact fat cells and fat cell membranes (2,3). The effect of trypsin digestion (Table IV) is also similar to the effect on fat cells (3).

TABLE IV. Effects of NaCl and Enzymic Digestions of Liver Membranes on the Specific Binding of ^{125}I-Insulin

Liver membranes (0.5 mg per ml) were incubated for 40 minutes at 37° in KRB buffer, 1% albumin, containing phospholipase C (50 μg per ml), phospholipase A (70 μg per ml), trypsin (0.1 mg per ml), and in some cases $CaCl_2$ (10 mM). The enzymes were removed by centrifugation, suspension in buffer, and re-centrifugation. Membrane suspensions (60 μg per ml) were tested for specific ^{125}I-insulin (1.1×10^{-10} M) binding as described in Figure 1. Phospholipase A effects are only observed in the presence of $CaCl_2$; Ca^{++} has no effect on phospholipase C digestion. These effects will be described in more detail elsewhere (3).

Membrane Treatment	Specific Binding of ^{125}I-Insulin
	nmoles x 10^5/mg protein
No enzymic digestion	5.1 ± 0.2
Phospholipase C, 5 μg per ml	9.4 ± 0.3
50 μg per ml	9.8 ± 0.2
Phospholipase A, 20 μg per ml (no Ca^{++})	5.2 ± 0.3
20 μg per ml (Ca^{++})	7.1 ± 0.2
Trypsin, 0.1 mg per ml	0.3 ± 0.1
NaCl, 0.5 M	9.1 ± 0.3
1.0 M	12.4 ± 0.1
2.0 M	19.6 ± 0.2
3.0 M	10.7 ± 0.3

DISCUSSION. Binding of iodoinsulin to liver membranes has been reported recently (7,8), but the properties of the binding have not been characterized. The insulin-membrane interactions presented in this report almost certainly

represent the specific interaction between insulin and the liver cell receptor for this hormone. Specific binding is a saturable process and competition for binding is observed only with biologically active derivatives of insulin. The interaction is a simple, reversible process having a dissociation constant (K_d, 7×10^{-11} M) near the physiologic concentration of insulin in serum (9).

There are striking similarities in the properties (kinetics, affinity and effects of hormone analogs, enzymes and ionic strength) of the interaction of insulin with liver and with intact fat cells and fat cell membranes. There is little doubt that insulin-receptor interactions are being studied in the experiments on intact fat cells (2). The present data suggest that the insulin receptors of liver and adipose tissue may be similar, if not identical, chemical structures.

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