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40. Supported by NIH grant A1 15387 and by United States-Israel Binational Science Foundation grant 1786.

11 August 1980

Hormone Binding Alters the Conformation of the Insulin Receptor

Abstract. *Fat cells or fat cell membranes were briefly subjected to mild proteolysis under conditions where insulin receptors were either free or bound to ^{125}I-labeled insulin. When receptors were then affinity-labeled to visualize the effects of this treatment, it was observed that receptors that had been occupied by ligand during proteolysis exhibited greater rates of degradation than unoccupied receptors. These results demonstrate that insulin-receptor interaction induces a change in receptor structure that may be related to signal transmission.*

The binding of insulin to specific receptors on the membranes of target cells such as adipocytes initiates a broad range of well-known metabolic effects (*1–5*). However, the molecular events that occur after binding and before the onset of the biological response remain undescribed. Presumably, insulin binding triggers a structural change in the receptor that results in the generation of the transmembrane signal. We have recently developed affinity cross-linking techniques that allow the identification of insulin receptors in cells and membranes (*6, 7*). These procedures have provided information on receptor subunit composition, indicating that the subunit exists in native membranes as a disulfide-linked complex containing a total of four subunits (*7, 8*). Purification of the [^{125}I]insulin-receptor complex by ligand-directed affinity chromatography has also been achieved (*9*). By combining the affinity cross-linking approach with biological assays after limited proteolytic digestion of adipocytes, we have also been able to correlate aspects of insulin receptor structure with biological functions (*10*). In the present experiments, we sought to extend the combined approach of limited tryptic digestion with affinity cross-linking in an attempt to detect differences in proteolytic sensitivity between occupied and unoccupied insulin receptors.

If the binding of insulin to its receptor results in a conformational change or if insulin binding stabilizes a preexisting conformational state of the receptor, we reasoned that differences in proteolytic susceptibility of the receptor in the occupied and unoccupied states may be detectable. Adipocytes were prepared by subjecting tissue obtained from the epididymal fat pads of male Sprague-Dawley rats (150 to 200 g) to collagenase digestion (*11*). The cells were then briefly treated with trypsin either before or during the last 2 minutes of a 30-minute incubation period with [^{125}I]insulin. The adipocytes were cooled to 15°C, and the cross-linking agent disuccinimidyl suberate was added in order to affinity-label the receptor. The cells were washed and disrupted, and a crude membrane fraction was prepared by centrifugation at 30,000*g* for 30 minutes. Figure 1 shows data for such fat cell membranes derived from adipocytes that were briefly exposed to trypsin in the absence or presence of receptor-bound [^{125}I]insulin and then subjected to electrophoresis (*12*) and autoradiography. At both times of trypsin treatment, the affinity-labeled receptor subunit, M_r 125,000, was less abundant when the insulin was receptor-bound than when the receptor was free during proteolysis, indicating a more complete tryptic digestion in the presence of bound insulin.

As previously observed, several receptor fragments that remain bound to the cell membrane are generated under these experimental conditions. The major receptor fragments are more abundant when proteolysis is carried out in

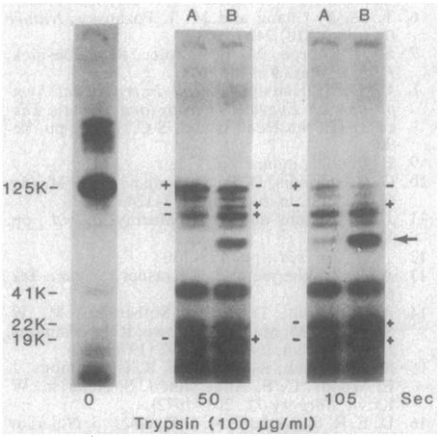

Fig. 1. Tryptic digestion of the adipocyte insulin receptor in the presence or absence of receptor-bound hormone. Fat cells in Krebs-Ringer phosphate buffer, *p*H 7.4, containing bovine serum albumin (20 mg/ml) were treated with trypsin (100 μg/ml) for the times indicated either before (A) or during (B) the last 2 minutes of a 30-minute incubation period at 24°C with $5 \times 10^{-9}M$ [^{125}I]insulin. The cells were then cooled to 15°C and the receptor and fragments thereof were affinity-labeled by cross-linking bound [^{125}I]insulin with 0.5 m*M* disuccinimidyl suberate (*6*). A crude membrane fraction was obtained by centrifugation of disrupted cells. The membranes were solubilized in sodium dodecyl sulfate and subjected to electrophoresis (*12*) on a 5 to 15 percent acrylamide gradient gel and autoradiography as described (*6*). The molecular weights ($\times 10^{-3}$) of the receptor subunit M_r 125,000 and its major low-molecular-weight fragments are indicated on the left of the figure.

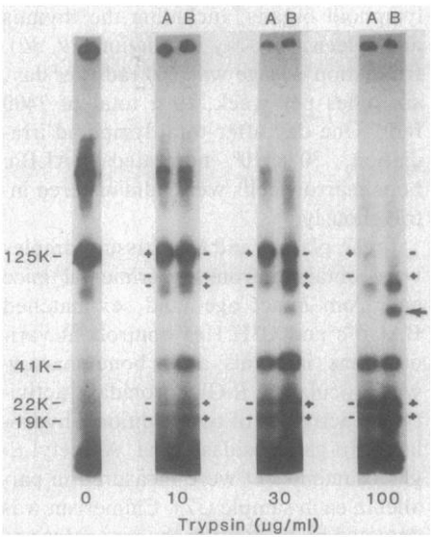

Fig. 2. Tryptic digestion of the insulin receptor in plasma membranes isolated from fat cells. A purified plasma membrane preparation was obtained from adipocytes by the procedure of Kono *et al.* (*13*). These membranes were suspended in Krebs-Ringer phosphate buffer, *p*H 7.4, containing bovine serum albumin (10 mg/ml), and were digested with the trypsin concentrations indicated either before (A) or during (B) the last 2 minutes of a 30-minute incubation period at 24°C with $5 \times 10^{-9}M$ [^{125}I]insulin. Affinity labeling was accomplished by using 0.5 m*M* disuccinimidyl suberate as described in the text and elsewhere (*6, 7*). Depicted is an autoradiograph of 5 to 15 percent acrylamide gradient gel prepared after solubilization of the labeled membranes in sodium dodecyl sulfate. The molecular weight of the intact receptor subunit M_r 125,000 and the major tryptic fragments are given on the left of the figure.

the presence of bound hormone rather than when the receptor is unoccupied. Relative abundance is denoted in Fig. 1 by plus and minus signs, and has been substantiated by direct measurement of radioactivity in gel slices. The species indicated by the arrow represents bovine serum albumin linked to [^{125}I]insulin fragments. This species is seen only in the presence of both trypsin and [^{125}I]insulin. During the brief period of exposure to trypsin (see Fig. 1) less than 10 percent of bound insulin dissociates from the receptor; this was shown by us (*6*) and others (*13*) for both intact adipocytes and isolated plasma membranes from fat cells.

We conducted similar experiments using isolated plasma membranes rather than intact cells. We prepared purified plasma membranes essentially according to Kono *et al.* (*14*). After subjecting the fat cells to collagenase digestion as above, we washed them twice in 10 m*M* tris and 1 m*M* EDTA buffer, *p*H 7.4, containing 0.25*M* sucrose. The cells were then homogenized in the same buffer with a Potter-Elvehjam homogenizer; the packed cell to buffer ratio was approximately 4 to 1. The broken cells were then centrifuged for 2 minutes at 13,000*g*. The supernatant was retained and centrifuged for 60 minutes at 130,000*g*. The pellet from this spin was resuspended in 10 m*M* tris and 1 m*M* EDTA, pH 7.4, containing 0.25*M* sucrose, and a purified plasma membrane fraction was obtained from a 15 to 45 percent linear sucrose gradient centrifuged for 1 hour at 27,000 rev/min in a SW27 rotor. The plasma membrane equilibrated at about 30 percent sucrose. The fraction obtained from the gradient was diluted in 10 m*M* tris and 1 m*M* EDTA, *p*H 7.4, centrifuged for 30 minutes at 30,000*g*, and resuspended in the same buffer for storage at −20°C.

The membranes were subjected to mild trypsinization before or during incubation with [^{125}I]insulin before they were cooled to 0°C and washed once by centrifugation. They were resuspended in Krebs-Ringer phosphate buffer, *p*H 7.4, containing the cross-linking agent, and the labeled receptor and its fragments were resolved on dodecyl sulfate polyacrylamide gels (Fig. 2). As in the case with intact adipocytes, the occupied receptor exhibited a greater sensitivity to proteolysis than the unoccupied receptor. This was more evident at concentrations of 10 and 30 μg of trypsin per milliliter and less so at 100 μg/ml. Conversely, the receptor fragments generated by trypsin were more abundant when proteolysis was performed in the presence rather than absence of bound [^{125}I]insulin.

In all five experiments with intact adipocytes and three experiments with isolated membranes the results were qualitatively similar and the opposite of what one would expect if insulin binding merely protected the receptor from proteolysis by masking potential cleavage sites. However, the low-molecular-weight receptor fragments do appear to be protected. These are observed in greater abundance when proteolysis is carried out in the presence of [^{125}I]insulin (Figs. 1 and 2) and cannot be accounted for simply by the increased fragmentation of the native receptor (for example, see Fig. 2 trypsin at 100 μg/ml). These results are consistent with an insulin-induced conformation change in the receptor which renders a cleavage site peripheral to the binding area more accessible to trypsin. The receptor site that is covalently linked to [^{125}I]insulin by cross-linking is itself protected by occupied hormone although this protective effect may also be a result of a conformational change in the receptor. An alternative hypothesis is that the insulin receptor exists in two or more conformational states in equilibrium and that hormone binding stabilizes the form which causes a biological response. According to this model, the M_r 125,000 subunit in the stabilized receptor state is more readily attacked by trypsin.

Hormone action in general may be initiated by a hormone-induced conformational change occurring after hormone-receptor binding. Thus an agonist-induced conformational change has been proposed for the β-adrenergic receptor of the turkey erythrocyte (*15*), for the cholera toxin–labeled guanine nucleotide regulatory protein coupled to the β-receptor in pigeon erythrocyte membranes (*16*), and for the acetylcholine receptor from *Torpedo californicum* (*17*). Our data further support this notion that hormone-receptor interaction involves a conformational change upon hormone binding. It is not known if this structural alteration is a prerequisite for transmembrane signaling.

The differential sensitivity of occupied as opposed to unoccupied insulin receptor to proteolytic cleavage may also be useful to probe hormone-receptor interaction in some physiological states that exhibit insulin resistance. A variety of insulin resistant conditions occur in man and rodents. If a ligand-induced conformational change is required for hormone action, it may be possible to correlate a blunted biological response in certain cases of insulin resistance with receptors that lack a differential sensitivity to trypsin in the presence and absence of bound hormone.

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29 May 1980; revised 4 August 1980