Affinity change of the adipocyte receptor fails to alter insulin-stimulated glucose transport

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Occupancy increased the affinity of the insulin receptor of the adipocyte. During the affinity change the half-maximal sensitivity of glucose transport to insulin stimulation was unaltered. Decreased maximum response of transport only occurred after the affinity change. There was not a simple relationship between receptor affinity and insulin stimulation of glucose transport in the adipocyte.

The binding of peptide hormones, including insulin, to specific receptors on the surface of target cells leads to biological responses (Czech, 1980). However, chronic hormone exposure frequently diminishes the responsiveness of the target cell to subsequent stimulation (Catt *et al.*, 1979). A relationship has often been observed between target-tissue sensitivity to stimulation by hormones and the state of receptor affinity. The most generally accepted description of this was first proposed by Katz & Thesleff (1957). It was later found that receptor occupancy induces a change of the binding-site affinity in many peptide hormone and neurotransmitter (Donner, 1980, and references cited therein) systems. Occupancy alters the equilibrium between affinity states, increasing the proportion of high-affinity sites. The insulin receptors of both the hepatocyte (Donner & Corin, 1980; Huber *et al.*, 1980; McCaleb & Donner, 1981) and adipocyte (Olefsky *et al.*, 1979; Häring *et al.*, 1981) are comparable since occupancy converts part of the receptor population to a high-affinity state. The similar characteristics of the insulin and other receptor systems led us to test if a relationship exists between insulin-receptor affinity and the sensitivity of the glucose-transport system of the adipocyte.

Experimental

Materials

Male Sprague–Dawley rats (175–275 g) were purchased from Charles River Laboratories and fed Abbreviation used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethane sulphonic acid.

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Purina laboratory chow *ad libitum*. Other materials and their sources were: bovine serum albumin fraction V, Miles; Parker medium 199, Grand Island Biological; chloramine-T, Matheson, Coleman and Bell; Sephadex G-50 fine grade, Pharmacia; Na¹²⁵I (carrier free) and 2-deoxy-D-[1-¹⁴C]glucose (5 μ Ci/mmol), New England Nuclear; dionylphthalate oil, ICN Pharmaceuticals; cytochalasin B (B grade), Calbiochem; collagenase (146 units/mg), Worthington; Hepes (25 mM in Krebs–Ringer buffer, 1.26 mM in Medium 199 buffer), Eastman Kodak. All other materials were obtained from Sigma or Fisher Scientific and were of analytical grade.

Methods

Isolated adipocytes were obtained from epididymal fat pads according to the method of Rodbell (1964) except that Medium 199 was used in place of sodium bicarbonate buffer (Smith, 1970). Adipocytes were incubated in Krebs–Ringer buffer/1% (w/v) bovine serum albumin under an O₂/CO₂ (19:1) atmosphere at 37°C. Glucose transport was assayed by measuring uptake of 2-deoxy-D-[1-¹⁴C]glucose by adipocytes according to the method of Gliemann *et al.* (1972) as modified by Olefsky (1975). Transport was terminated by the oil-flotation centrifugation method after 4 min of assay. Radioactivity accumulated in the presence of cytochalasin B (50 μ M) was subtracted from the basal- and insulin-stimulated transport to account for non-specific uptake.

Monocomponent, immunocompetent ¹²⁵I-labelled insulin (specific radioactivity 200 Ci/g) was iodinated by the method of Greenwood *et al.* (1963) and Lesniak *et al.* (1973). Specific binding of ¹²⁵I-labelled insulin (0.8 nM) to fat-cells (2.1 $\times 10^5$ cells/ml) was determined by the method of oil flotation (Gliemann

et al., 1972). Hormone-binding data were fitted to appropriate mathematical expressions by using non-linear least-squares curve-fitting techniques (Donner *et al.*, 1980).

Results and discussion

Fig. 1 shows that, as the time of association between ^{125}I -labelled insulin and adipocytes was lengthened from 8 to 45 min, the half-time of hormone dissociation increased from 4 to 10 min. These observations confirm similar data previously reported by Olefsky *et al.* (1979) and Häring *et al.* (1981). Additionally, similar results were obtained using partially purified plasma membranes from the adipocyte (Olefsky *et al.*, 1979). The diminished rate of dissociation was therefore due to a change of insulin-receptor affinity (Olefsky *et al.*, 1979) as well as to internalization of radioactivity (Suzuki & Kono, 1979). Similar observations made with rat hepatic tissue (Donner & Corin, 1980; Huber *et al.*, 1980; McCaleb & Donner, 1981) suggest that an increase of insulin-receptor affinity after occupancy may be a general occurrence in target tissues for this hormone.

The rate of uptake of 2-deoxy-D-[1- ^{14}C]glucose by adipocytes was linear from 3 to 15 min during the transport assay (results not shown). Approx. 93% of the 2-deoxy-D-[1- ^{14}C]glucose uptake, which was stimulated by insulin, could be inhibited by $50\text{ }\mu\text{M}$ -cytochalasin B. Basal uptake of 2-deoxy-D-[1- ^{14}C]glucose was not significantly altered by a 1 h

incubation of adipocytes. There was variability in the amount of basal uptake from day to day. However, insulin generally increased the uptake of 2-deoxy-D-[1- ^{14}C]glucose 2–3-fold above basal levels.

The stimulation of glucose transport by increasing concentrations of insulin was examined after 8 and 45 min of incubation. These times were equivalent to those shown above to alter the affinity of part of the receptor pool. As seen in Fig. 2, the ability of insulin to stimulate glucose transport in the adipocyte was not affected by the lengthened time of exposure to hormone. Thus, a simple relationship was not observed between the retention of insulin by the adipocyte (Fig. 1) and the sensitivity of glucose transport to insulin stimulation (Fig. 2).

We confirm that preincubation of adipocytes with or without insulin for up to 2 h does not change the half-maximal concentration of the dose-response curve from a subsequent incubation with insulin (results not shown; Marshall & Olefsky, 1980). There was a decrease in the maximal response after 1 h of pre-exposure to insulin. This may have resulted from a decrease in the number of insulin receptors on the cell surface (Roth *et al.*, 1975), a post-receptor defect (Crettaz & Jeanrenaud, 1980), loss of cell viability or depletion of ATP reserves (Kono *et al.*, 1977). In any case, the decrease of maximal

Fig. 1. Decrease of insulin dissociation by lengthened association time

Dissociation (37°C) of ^{125}I -labelled insulin initiated after 8 (●) or 45 (○) min of association at 37°C is shown. Hormone bound at any time is expressed as a percentage of the quantity at 0 min of dissociation. These values were 370×10^{-11} or 230×10^{-11} pmol/cell after 8 or 45 min of association, respectively.

Fig. 2. Stimulation of glucose transport by insulin. Insulin-dependent stimulation of 2-deoxy-D-[1- ^{14}C]glucose transport in adipocytes after 8 (●) or 45 (○) min of incubation is shown. The uptake of 2-deoxy-D-[1- ^{14}C]glucose is expressed as a percentage of the maximum quantity stimulated above basal ($51\text{ pmol/4 min per } 10^5\text{ cells}$) levels by 3.3 nM -insulin. The averages of values from two experiments were 104 or $109\text{ pmol/4 min per } 10^5\text{ cells}$ after 8 or 45 min of incubation, respectively.

response occurred only well after a significant portion of the receptor population had undergone the change of affinity. Therefore, the increase of receptor affinity did not occur at the same time as the decrease in the maximal stimulation of glucose transport by insulin.

There was not a positive correlation between changes of receptor affinity and cellular responsiveness in the adipocyte. Whether such a relationship exists in other target cells for insulin is not known. A number of explanations may be offered to rationalize these observations. We (unpublished results) and others (Olefsky *et al.*, 1979) have found that only about one-third of the total receptor population of the adipocyte is converted to a high-affinity state by extended periods of occupancy. This is in marked contrast with the hepatocyte, in which virtually all of the receptors can be stabilized as a high-affinity complex (Donner & Corin, 1980; McCaleb & Donner, 1981). Since only 5–10% of the total number of adipocyte receptors for insulin need to be occupied to produce maximum stimulation of glucose transport (Gammeltoft & Gliemann, 1973; Olefsky, 1975), a sufficient number of low-affinity receptors may have been present to produce full response.

Livingston *et al.* (1978) observed a change of sensitivity after a 17h incubation of adipose tissue with insulin. It was suggested that this observation might be consistent with either receptor or post-receptor defects in the insulin receptor-effector system. In fact, many recent studies (Crettaz & Jeanrenaud, 1980) have suggested that a lesion not directly related to the insulin receptor itself may be responsible for loss of cellular responsiveness. The progressive internalization of hormone (Suzuki & Kono, 1979; Marshall & Olefsky, 1979), uncoupling of receptors from the transduction event (Pillion *et al.*, 1980), hormone degradation (Gliemann & Sonne, 1978), up-regulation of insulin receptor number (Corin & Donner, 1981) or the mobilization of glucose transport units (Karnieli *et al.*, 1981; Kono *et al.*, 1981) may mask the observation of a relationship between receptor affinity and cellular responsiveness. Given the number of factors that may modulate insulin action, this relationship, if it does exist, may be difficult to demonstrate in the adipocyte.

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