DECREASED INSULIN SENSITIVITY DUE TO A POSTRECEPTOR DEFECT AS A CONSEQUENCE OF ATP-DEFICIENCY IN FAT CELLS

H. U. HÄRING, F. RINNINGER and W. KEMMLER
Institut für Diabetesforschung, Kölner Platz 1, 8000 München 40, FRG

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1. Introduction

The stimulatory effect of insulin on glucose transport in fat cells and muscle requires cellular energy [1–5]. In fat cells with an ATP level of ~10% of normal the transmission of the insulin signal from the receptor to the glucose transport system is completely blocked [1–4], whereas an ATP level of 30–50% of normal is sufficient to preserve the full response of the transport system to high concentrations of insulin [1,4]; only the velocity of the signal transmission is considerably slower in these cells [4]. Thus a submaximal decrease of the cellular ATP does not change the responsiveness [6] of the fat cell; it is, however, not yet known if a change in the sensitivity [6] occurs under these conditions as in the earlier studies only the effect of high doses of insulin and no dose–response relations were tested. Here, we therefore investigated the dose–response relation of insulin binding and insulin action on 3-*O*-methylglucose transport in cells with an ~50% decreased cellular ATP level.

2. Materials and methods

Male Sprague Dawley rats fed ad libitum (180–200 g body wt) were used. Fat cells were prepared according to [7]. All incubations were performed in Krebs–Ringer–Hepes buffer (pH 7.4), containing 2.5 g/dl crystalline bovine serum albumin. Adipocyte counts were performed in triplicate in a Neubauer chamber.

2.1. *Binding studies*

Fat cells ($4.5–5.5 \times 10^5$/ml) were incubated in polyethylene vials at 37°C. Labeled insulin (^{125}I-insulin, spec. act. 250 μCi/μg, Novo Industri, Bagsvaerd) at 10 μU/ml alone or together with increasing concentrations of unlabeled insulin (see legend fig.1A) were added at time 0. After 20 min, 400 μl aliquots were transferred into polyethylene centrifuge tubes in a high-speed table centrifuge. Separation of cells and medium was performed by centrifugation through dinonylphtalate [8]. The tube was cut at the oil layer and the radioactivity of the cell layer was determined. To determine the unspecific binding an excess of unlabeled insulin (1 U/ml) was added together with the labeled insulin.

2.2. *3-O-Methylglucose transport*

3-*O*-Methylglucose transport was determined by the method in [9] as described [4]. Fat cells were preincubated for 20 min or 30 min in the absence or presence of insulin (5 μU–1000 μU/ml). Aliquots (100 μl) of a concentrated cell suspension (5×10^6 cells/ml) were drawn together with 200 μl 3-*O*-methylglucose (final conc. 0.5 mM, 0.1 μCi 3-*O*-methyl-D-[^{14}C]glucose from the Radiochemical Centre, Amersham, as tracer) into a mixing pipette (Gilson, Medical Electronics, France). After 5 s the uptake was stopped by dilution of the cells in 5 ml cold NaCl solution (0.9 g/dl) containing phloretin (1 mM). Subsequently cells and medium were separated by centrifugation through silicone oil at $1000 \times g$ for 30 s. The cell layer was removed from the oil by a pipette and added to scintillation fluid for the determination of the radioactivity. To obtain a value for the extracellularly trapped 3-*O*-methylglucose and 3-*O*-methylglucose taken up by diffusion the uptake was determined in the presence of 1 mM phloretin and all other uptake values were corrected for this amount.

To reduce the cellular ATP level cells were prein-

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cubated for 5 min with dinitrophenol (DNP) (0.35 mM) or KCN (1 mM); at these inhibitor concentrations an ~50% decrease of the cellular ATP level was seen in [4]. Here, the effect of the inhibitors was controlled in selected experiments by a luciferase assay [10].

3. Results

Fig.1B shows the dose–response curve of insulin action on the 3-*O*-methylglucose uptake in fat cells which are pretreated with DNP (0.35 mM) and control cells. In these cells, the half-maximal stimulation of the glucose-transport system is obtained at ~70 μU insulin/ml, whereas the half-maximal stimulation in the control cells is obtained at ~15 μU insulin/ml.

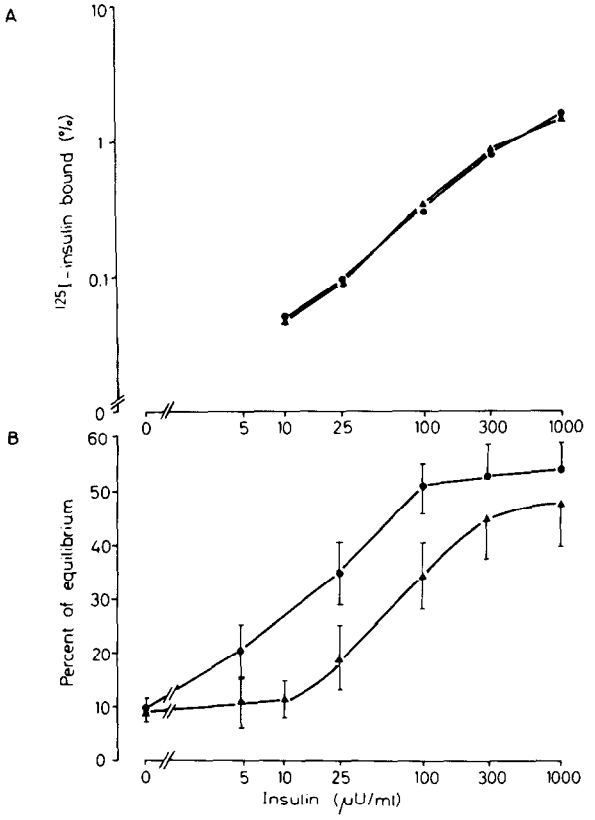

Fig.1. (A) Binding of ^{125}I-insulin to 0.35 mM DNP-treated cells (▲) and control cells (●). Values represent the mean of 2 expt. (B) Dose–response curve of the insulin effect on the 3-*O*-methylglucose transport in 0.35 mM DNP-treated cells (▲) and control cells (●). Transport values are expressed as fraction of the equilibrium space of the fat cells filled in 5 s. The values represent the mean ± SEM of 6 expt.

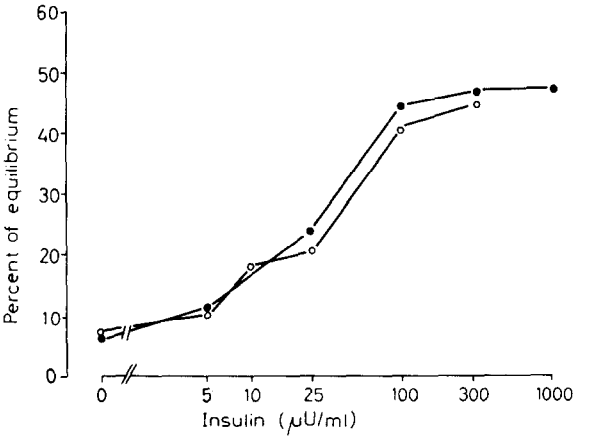

Fig.2. Reversibility of the effect of DNP. Dose–response curve of the insulin effect on the 3-*O*-methylglucose transport in control cells (●) and 0.35 mM DNP-treated cells which were subsequently washed free of DNP (○). The points represent values of 1 expt.

The maximal effects of insulin are not significantly different in both cell types. Thus the DNP-treated cells show a decrease of the insulin sensitivity but, as also shown in [4], an unchanged responsiveness. The chance in the sensitivity must be solely caused by an alteration of the post-receptor signal transmission as no differences of the insulin binding (fig.1A) between both cell types are evident. The effect of DNP is completely reversible as can be seen from fig.2. One representative experiment is shown where cells were treated with DNP as in the experiments of fig.1; sub-

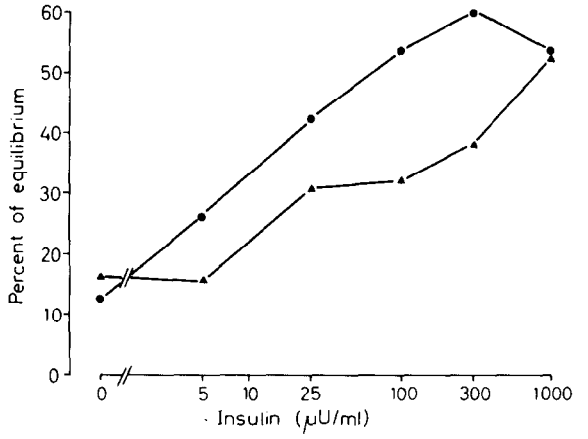

Fig.3. Dose–response curve of the insulin effect on the 3-*O*-methylglucose transport in 1 mM KCN-treated cells (▲) and control (●) cells. The values are the mean of 2 expt.

sequently the cells were washed free of DNP. In this experiment the ATP level was determined. It fell to ~50% after addition of DNP and rose after washing of the cells again up to the value of control cells. This is in agreement with [1,4]. Correspondingly full sensitivity of the cells was restored.

Fig.3 shows that treatment of the cells with KCN (1 mM) leads to a similar shift of the dose–response curve of insulin action as observed in the DNP-treated cells.

4. Discussion

The data show that an ~50% decreased cellular ATP-level causes an insensitivity of the fat cell due to an alteration of the insulin signal transmission. It is, however, interesting that the reduced efficiency of the postreceptor insulin signal can be compensated on the receptor level, i.e., by increasing the receptor occupancy.

A rightward shift of the dose–response curve without a significant change of the basal and maximal level is a pattern of resistance which is considered indicative of alterations on the prereceptor or receptor level, while postreceptor alterations are expressed as changes in the responsiveness of the cell as outlined in [6]. The present data show that this interpretation is not valid in all cases of insulin resistance.

It is therefore not possible to localize a cellular defect causing insulin resistance solely from the shape of a dose–response curve. Furthermore, the data indicate a thus far unknown function of spare receptors. The insulin binding to spare receptors can not only overcome changes on the receptor level, i.e., reduced receptor number, they can also compensate defects in the postreceptor signal transmission.

It is difficult to interpret the results in terms of molecular events participitating in the signal transmission as there is at present no precise knowledge of the mechanism of the coupling and of the nature of the ATP-dependent step in this process. Under the influence of insulin a translocation of glucose carriers from intracellular membranes to the plasma membrane occurs [11,12]. However, this may not be the exclusive mechanism of insulin activation of the glucose transport system. Many of our observations on the characteristics of the receptor glucose transport coupling process [4,14,15] can be conclusively integrated into the translocation model. It was seen that the ATP-dependent step in insulin action on glucose transport is the transmission of the insulin signal from the receptor to the transport system, whereas the initial binding reaction and the activation state of the transport system are not influenced by the cellular ATP-level. Furthermore the velocity of the signal-transmitting process is critically ATP-dependent. These data show that also the efficiency of this process is dependent on the actual ATP-level and that inefficiency caused by lack of energy can be compensated by increasing the number of signals produced by occupied receptors.

In experimental hemorrhagic shock of rats the muscular ATP-content can fall to 50% of normal [16]. Although data for other tissues are not known to us it might be speculated that an insulin insensitivity as seen here may occur in other clinical situations of reduced blood circulation and that this insulin insensitivity may apply to other anabolic effects of the hormone. Trophic defects of tissues in patients with vascular disease might be partly due to an insulin resistance of the type observed in these experiments; therapeutically high doses of insulin could overcome this defect since sensitivity is altered and not the responsiveness.

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