Vol. 122, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 18, 1984 Pages 97-102

EFFECT OF GLUCOSE ON BETA-ADRENERGIC INDUCED DOWNREGULATION OF INSULIN RECEPTOR BINDING IN HUMAN FAT CELLS

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Received May 10, 1984

SUMMARY. The effect of beta-adrenergic stimulation on specific insulin binding to isolated human fat cells was investigated at 24°C and at 37°C. In the abscense of glucose isoprenaline caused a 40% decrease in high affinity insulin binding at both temperatures. At 37°C the reduction in binding was completely offset by the addition of glucose to the medium. A maximum effect of glucose occured at 5 mmol/l. At 24°C, however, there was no effect of glucose on insulin binding. The effects of glucose and isoprenaline on insulin binding were not related to the lipolytic activities these two agents. In conclusion , low amounts of glucose prevent catecholamine induced down-regulation of insulin receptor binding in human fat cells at physiological temperature.

It has become increasingly apparent that the binding of a certain hormone to its receptor on a target cell can change the binding of another hormone to the same cell. The first hormone may thereby regulate the physiological response of cells to the second hormone. Recent studies have shown that catecholamines reduce insulin receptor binding to isolated rat adipose cells by means of beta-adrenergic receptors (1,2). This may explain why catecholamines inhibit the peripheral action of insulin and thereby cause insulin resistance (3,4). In the present study we investigated the effect of beta-adrenergic stimulation on specific insulin binding to isolated human fat cells.

MATERIALS AND METHODS

Subcutaneous adipose tissue was obtained at the start of elective benign surgery on normalweight patients of both sexes aged 30-60 years. The subjects fasted overnight and were only

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0006-291X/84 $1.50

given saline intravenously prior to the removal of fat. Informed consent was obtained. Isolated fat cells (5) constituting 4% of a total volume of 0.2 ml were incubated in triplicate for 2 h at 37°C or 24° C. The medium consisted of Krebs-Henseleit bicarbonate buffer (pH 7.4) with albumin (40 g/l), bacitracin (2 g/l), glucose (0-10 mmol/l). Isoprenaline (0 or 6 µmol/l), mono ^{125}I-Tyr(A 14)-insulin (0.05 nmol/l) and various concentrations of unlabelled insulin (0-50 nmol/l). A mixture of O_2:CO_2 (95:5) was used as the gas phase. Cell associated radioactivity was determined. The reaction was terminated by adding 2.5 ml of ice-cold saline and rapidly centrifuging the cells through 0.7 ml of silicone oil. The data were corrected for non-specific binding (about 4%), which was measured in the presence of 0.2 mmol/l of insulin. Insulin degradation was negligible and a steady state of insulin binding was reached after 40 min and maintained throughout the incubation period (6). The coefficient of variance for insulin binding was 7% in one subject. In some experiments an aliquote of the medium was removed immediately after the incubation period for the determination of glycerol (7).

RESULTS AND DISCUSSION

When human fat cells were incubated in the absence of glucose (Fig. 1) at 24°C or 37°C the addition of 6 µmol/l of isoprenaline was accompanied by a 40% and significant decrease in insulin receptor binding which occurred at low, physiological concentrations of insulin (< 1 nmol/l). At both temperatures isoprenaline had no effect on insulin binding occurring at high insulin concentrations (> 2 nmol/l). When fat cells were incubated with 10 mmol/l of glucose (Fig. 1, upper part), the addition of isoprenaline did not influence insulin binding at 37°C. However, at 24°C isoprenaline inhibited insulin binding in a similar fashion with as without glucose (Fig. 1, bottom part). When binding data were transformed using Scathcard' s method (8) curvilinear plots were obtained. This indicates that insulin binds to one class of receptors with negative cooperativity (9) or that it binds to discrete high and low affinity receptors (10). It is not possible at present to distinguish between these two models of insulin receptor binding. The curve for fat cells incubated at 37°C with isoprenaline but without glucose started at a lower position than the other two curves in the Scatchard graph. All three curves, however, intercepted the abscissa at the same point. This indicates that iso-

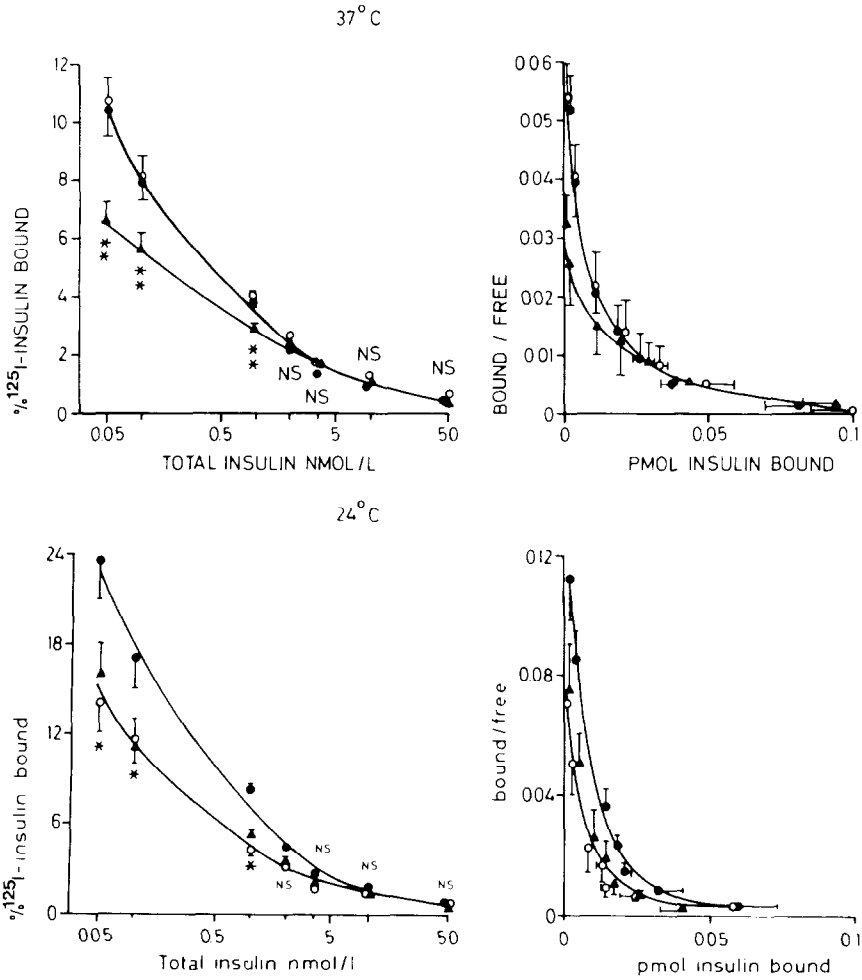

Figure 1. Effect of isoprenaline and glucose on specific insulin receptor binding. Isolated fat cells were incubated at 37°C (upper panel) and at 24°C (lower panel) for 2 h. The binding data represent mean ± standard error of the mean (SE) of 7 subjects. They are presented as competition plots (left) and Scatchard plots (right). Solid circles represent control cells, open circles represent isoprenaline and glucose and solid triangles represent isoprenaline alone. Isoprenaline and control experiments were compared statistically using Wilcoxon' s paired test. *= $p<0.05$, **= $p<0.02$, NS = not significant. Binding data are normalized for 5 x 10^5 fat cells/ml medium.

prenaline reduced the average insulin receptor affinity or lowered the number of high affinity insulin receptor number. The same conclusion could be reached with the Scatchard analysis of binding data obtained at 24°C.

In the experiments shown in Fig. 1 insulin, isoprenaline and glucose were added simultaneously to the fat cells. Similar re-

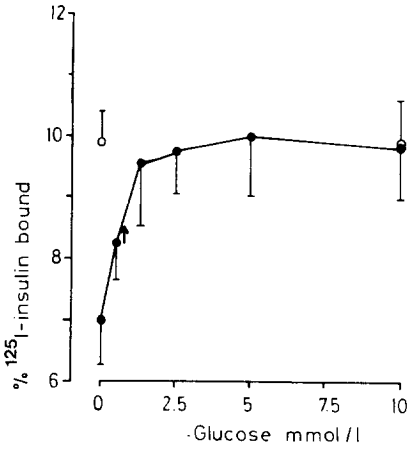

Figure 2. Effect of glucose on isoprenaline-induced insulin receptor binding. Human fat cells were incubated with 0.05 nmol/l of labelled insulin, with (solid circles) or without (open circles) 6 µmol/l of isoprenaline and with glucose added at various concentrations (0, 0.5, 1.25, 10 mmol/l). Specific insulin binding was determined. Data represent the mean ± SE of 5 subjects. The concentration of glucose giving the half maximum effect is indicated by an arrow. Other details are the same as to those in Fig 1.

sults were obtained, however, when fat cells were preincubated for 1 h in the presence of isoprenaline with and without glucose and insulin binding was determined after the cells were washed free of glucose and isoprenaline (data not shown).

When fat cells were incubated at 37°C in the presence of isoprenaline the addition of glucose at phsyiological concentrations stimulated tracer insulin receptor binding in a sensitive and dose-dependent way (Fig. 2). Maximum stimulation of insulin binding occurred at 5 mmol/l and a half-maximum effect occurred at a dose of only 0.75 mmol/l of glucose. When fat cells were incubated in the absence of isoprenaline, the addition of 10 mmol/l of glucose had no influence on tracer isulin binding (Fig.2). This suggests that glucose prevents the isoprenaline-induced inhibition of insulin binding and does not increase the insulin binding per se.

In theory isoprenaline could,by virtue of its ability to accumulate lipolytic endproducts, induce artifactual effects on insulin binding,particularly under conditions when glucose is absent.

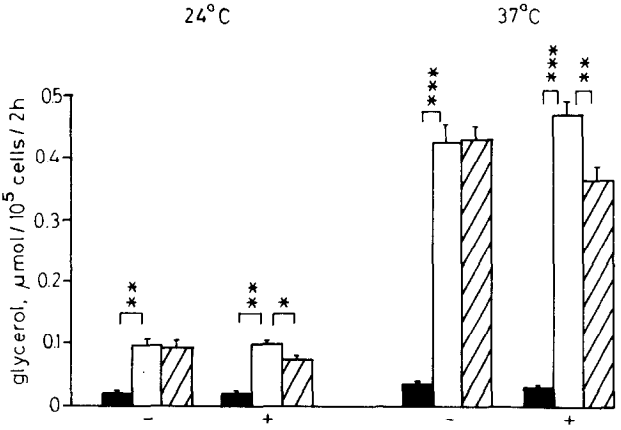

Figure 3. Lipolysis in human fat cells incubated at 24°C and 37°C. Glycerol release was determined after 2 h incubation. Fat cells were incubated with (+) or without (-) 10 mmol/l of glucose. Solid bars indicate no further addition. Open bars indicate 6 µmol/l of isoprenaline. Hatched bars indicate isoprenaline plus 1.5 pmol/ml of insulin. Data represents mean ± SE of 6 incubations. A statistical comparison was made between basal and isoprenaline experiments and between isoprenaline and isoprenaline plus insulin experiments. Other details are the same as to those in Fig 1.

In order to address this problem lipolysis (glycerol release)was determined under the same conditions as insulin binding. The results of the lipolysis experiments are depicted in Fig. 3. Isoprenaline induced lipolysis occurred at an approximately 4 times more rapid rate at 37°C than at 24°C. However, isoprenaline inhibited adipocyte insulin receptor binding in the same order of magnitude at both temperatures. In the presence of glucose insulin inhibited isoprenaline induced lipolysis in the same order of magnitude (about 25%) at both temperatures. On the other hand, glucose prevented isoprenaline induced downregulation of insulin binding only at 37°C (Fig. 1). These data indicate strongly that the effect of isoprenaline and glucose on insulin binding were not secondary to the action of these agents on lipolysis.

In conclusion, our study shows that isoprenaline, acting through beta-adrenergic receptors, rapidly inhibits high affinity insulin binding to human fat cell receptors. This effect of isoprenaline appears not to be temperature dependent. Furthermore, our data agrees with recent findings obtained with rat fat cells

(1,2). More important, however, is our observation that glucose, added in physiological concentrations, completely prevents isoprenaline induced downregulation of insulin receptor binding. This effect of glucose is clearly temperature dependent and may only occur at physiological temperature. At the present we have no clear explanation for how glucose prevents isoprenaline-induced downregulation of insulin binding. On the other hand our results are in marked contrast to the finding in the rat(1). There was no effect of glucose or of other carbohydrates on catecholamine-induced inhibition of insulin binding to rat fat cells(1). Thus there seem to be significant species differences in the interaction between catecholamines and insulin receptor binding. We have recently observed that glucose stimulates the antilipolytic effect of insulin in human fat cells (11). This also differs from the findings in rat. Evidently glucose plays an unique role in moderating various insulin actions in humans.

ACKNOWLEDGEMENTS. This study was supported by grants from the Swedish Medical Research Council, the Swedish Diabetes Association, Karolinska Institute and the Osterman, Folksam and Nordic Insulin Foundations.

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