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# Insulin Binding and Insulin Action in Rat Fat Cells after Adrenalectomy

H. Häring, C. Calle, A. Bug, R. Renner, K. D. Hepp, and W. Kemmler

Institut für Diabetesforschung, München, FRG

**Summary.** Insulin binding and the effect of insulin on the transport of 3-O-methylglucose, lipogenesis from glucose, glucose oxidation and lipolysis was studied in fat cells of adrenalectomised rats and of a control group of sham-operated rats. The serum insulin level of the adrenalectomised rats (0.7 ng/ml) was lower than that of the controls (1.6 ng/ml). In adrenalectomised rats as compared to sham-operated rats the insulin concentrations causing half-maximal effect were reduced by 50% in lipogenesis and antilipolysis and by 30% in glucose transport. The increase in sensitivity to submaximal insulin concentrations was not observed in glucose oxidation. The maximal responsiveness was unchanged in all test systems. The increase in sensitivity in three of the four studied insulin effects may be related to the 37% increase in the binding capacity of fat cells from adrenalectomised compared with sham-operated rats. The unchanged sensitivity with respect to glucose oxidation indicates possible post-receptor modulation. When adrenalectomised rats were substituted with either insulin or cortisol serum insulin levels were elevated above normal; however, the changes in the receptor were prevented in the cortisol supplemented rats and only partially in the insulin supplemented rats. The observation suggests, that the insulin receptor is regulated not only by the serum insulin level but also by cortisol.

**Key words:** Adrenalectomy, insulin receptor, cortisol, insulin effect, glucose transport, glucose oxidation. lipogenesis, antilipolysis.

An inverse relationship of the serum insulin levels and the number of insulin receptors is shown in vivo in different hyperinsulinaemic animal models [1–5] and also in patients with obesity [6, 7] or maturity onset diabetes [8, 9] showing relatively elevated insulin levels. In vitro the "down regulation" of the receptor by insulin has been demonstrated in lymphocytes [10] fat cells [11] and hepatocytes [12].

Greater than normal binding capacity was first demonstrated in the insulin deficient state using the model of the genetically diabetic Chinese hamster [13]. This observation has been confirmed in other animal models with decreased insulin levels e. g. fasting [14, 15] and with streptozotocin diabetes [16–19].

The relationship of insulin binding and insulin action, however, is very complex. Changes in binding do not always regulate the overall insulin sensitivity of the target tissue in the expected way, since the insulin sensitivity may also be influenced by altered insulin effector systems behind the receptor. Thus binding and action are not correlated in all situations of insulin deficiency [14–17].

In this study another animal model with decreased insulin levels, the adrenalectomised rat was evaluated. Hypersensitivity to insulin is shown in vivo in patients with adrenal insufficiency [20] and in adrenalectomised animals [21]. The in vitro data on the insulin sensitivity after adrenalectomy are somewhat contradictory [22–25]. In this study we have tried to answer the following questions:

1. Is there an inverse relationship between insulin levels and the number of insulin receptors in the adrenalectomised rat?
2. Is there a correspondence between the well-known hypersensitivity to insulin in vivo in the state of adrenal insufficiency and the action of insulin in vitro?
3. What is the relation of insulin binding and action in this animal model with decreased insulin levels?

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**Table 1.** Characteristics of animals and fat cells

| | Adrenalectomised rats (A) | Sham-operated rats (S) | Adrenalectomised rats with insulin treatment (AI) | Adrenalectomised rats with cortisol treatment (AC) |
|---|---|---|---|---|
| Serum insulin (ng/ml) | 0.7 ± 0.1 | 1.6 ± 0.2 | 9.1 ± 1.3 | 3.0 ± 0.2 |
| Serum glucose (mmol/l) | 6.9 ± 0.1 | 7.6 ± 0.15 | 6.3 ± 0.1 | 8.1 ± 0.1 |
| Body weight (g) | 194 ± 3 | 206 ± 2 | 206 ± 2 | 188 ± 5 |
| Fat cell diameter (μm) | 74 ± 2 | 77 ± 3 | 79 ± 3 | 72 ± 2 |
| Total lipid (mg/$10^5$ cells) | 11.4 ± 1.1 | 11.9 ± 1.3 | 11.6 ± 0.7 | 11.7 ± 1.3 |

The values for glucose and insulin are fed values and give the mean of 8 determinations ± SEM for each group. The sham-operated group was not pair-fed. The body weight was determined in 32 animals per group, the fat cell diameter in 300 cells of 8 animals per group and the total lipids in 8 animals per group. The differences in the insulin and glucose levels between all groups were statistically significant ($p \leq 0.001$). The body weights of group A and AC were statistically significant ($p \leq 0.001$) different from S and AI. All other differences were not statistically significant

## Materials and Methods

### *Materials*

[$^{125}$I]-beef insulin (specific activity 24–28 mCi/mg) and unlabelled monocomponent beef insulin (27 U/mg) were generous gifts from the Novo Company, Copenhagen; insulin (Depot Hoechst CR) and hydrocortisone were provided by Farbwerke Hoechst AG, Frankfurt/Main; bovine albumin was purchased from Behring Co., Marburg; 3-O-methyl-D-[$^{14}$C]-glucose (specific activity 50 mCi/mmol), D-[1-$^{14}$C]-glucose (specific activity 50 mCi/mmol) were from the Radiochemical Centre, Amersham, and DL-isopropylarterenol-HCl from Serva Co., Heidelberg. Szintigel from Roth Co., Karlsruhe, was used as scintillator. All other substances were reagent grade from Merck Co., Darmstadt.

### *Methods*

*Animals:* Five groups of male Wistar rats (weight 150–170 g) were studied: Group A, rats were adrenalectomised, fed ad libitum, and maintained on 0.154 mol/l saline; Group S, animals were sham-operated and fed ad libitum; Group SP, animals were sham-operated and pair-fed with group A rats during the test period; Group AC, adrenalectomised rats were substituted from the day of operation with cortisol (2 × 2.5 mg hydrocortisone/day/animal), maintained on 0.154 mol/l saline, and fed ad libitum. In the fifth group (AI) adrenalectomised rats received from the day of operation insulin (2 × 60 μg Depot Hoechst CR/day/animal), they were maintained on 50 g/l glucose in 0.154 mol/l saline and fed ad libitum. On the fifth day after surgery animals were sacrificed and isolated fat cells were prepared from the epididymal fat pads (26). In all assays a Krebs-Ringer-Hepes-buffer [27], pH 7.4, containing 2.5 g/100 ml bovine albumin was used.

*Insulin Binding:* Adipocytes (4–6 × $10^5$/ml, test volume 400 μl) were incubated with 50 μl of bovine [$^{125}$I]-insulin (2 ng) and 50 μl of unlabelled bovine insulin (0.2 ng to 20 μg) or 50 μl of buffer. After incubation for 45 min at 25 °C the adipocytes were separated from the medium by centrifugation through dinonylphthalate according to Gliemann et al. [28] and the cell layer was counted in a gamma counter. The amount of [$^{125}$I]-insulin bound in the presence of 20 μg of unlabelled insulin was considered as non specific binding. It ranged from 7–13% of the total [$^{124}$I]-insulin bound in the absence of unlabelled insulin and was subtracted from each value. The non specific binding was not different in the five groups.

*Determination of 3-0-methylglucose Transport:* Fat cells (5 × $10^6$/ml) were incubated at 37 °C. Samples of 100 μl were drawn together with 200 μl of 3-0-methylglucose (final concentration 500 μmol/l, 0.1 μCi 1-[$^{14}$C]-3-0-methylglucose as tracer) into a mixing pipet (Gilson Medical Electronics, Villiers-le-Bel, France). After time intervals of 5, 15, 25, 40, 60 and 120 seconds cells and medium were separated by centrifugation through dinonylphthalate [28]. The cell layer was dissolved in 200 μl of diphenylethylamine and counted in 10 ml scintillation fluid. The values thus obtained represent intracellular 3-0-methylglucose and 3-0-methylglucose in the extracellular waterspace trapped in the cell layer. The extracellular part was estimated by an extrapolation of the initial linear uptake kinetic to time zero and the values thus obtained on the ordinate were subtracted from each measurement. For the determination of the insulin effect fat cells were preincubated with insulin (60 pg–0.4 μg/ml) for 15 min at 37 °C. At this time point an equilibrium cf the insulin effect was reached at all insulin concentrations used.

*Antilipolysis:* Adipocytes (1 × $10^5$ in a test volume of 1 ml) were incubated for 1 h at 37 °C with isoproterenol $10^{-6}$ mol/l and insulin at the concentrations described for the individual experiments. The cells were separated from the medium by filtration through paper filters. The glycerol content of the medium was determined enzymatically [29].

*Glucose Oxidation and Lipogenesis:* Fat cells (4 × $10^4$ in a test volume of 1 ml) were incubated with 1 mmol/l glucose for 1 h at 37 °C. For glucose oxidation studies [$^{14}$C]-glucose, (0.1 μCi) and for lipogenesis 2-[$^3$H]-glucose (0.1 μCi) were added respectively as tracers. The incorporation of glucose into lipids was determined according to Moody et al. [30]. [$^{14}CO_2$]-production from 1-[$^{14}$C]-glucose was determined as described by Weitzel et al. [31]. Insulin was measured by radioimmunoassay with rat insulin standards according to Heding et al. [32]. The coefficient of variation of the assay was 7.6%, the sensitivity i. e. the smallest quantity of insulin that can be distinguished as significantly different from zero ( ± 2 SD) was 0.2 ng/ml. Total lipids were determined as described by Zöllner and Kirsch [33], and protein by the method of Lowry et al. [34]. Fat cell diameter was measured under a Zeiss microscope with an ocular micrometer [35]. Statistical analysis was performed using Student's t-test.

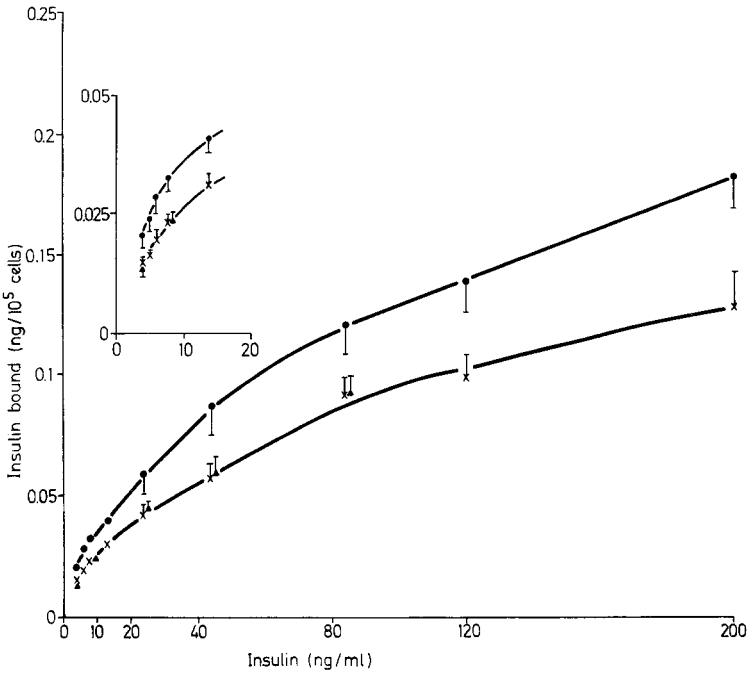


**Fig. 1.** Insulin binding to adipocytes of the adrenalectomised group (●), the sham-operated group (×), and the sham-operated pair-fed group (▲). The ordinate indicates the total insulin bound (radioactive labelled and unlabelled insulin), the abscissa the amount of total insulin in the incubation medium. Values for the adrenalectomised and the sham-operated group represent the mean of 8 experiments ± SEM, for the control-fed group the mean of 5 experiments. The differences between the adrenalectomised and the sham-operated group were statistically significant at all insulin concentrations used in the test ($p \leq 0.01$). The binding at lower concentrations is shown in the insert. For experimental details see Methods section

## Results

*Effect on Body Weight, Glucose and Insulin.* The effects of adrenalectomy, substitution with cortisol, and insulin treatment on body weight, serum glucose and serum insulin levels are shown on Table 1. The adrenalectomised animals were lighter because of decreased food intake [36], therefore, an additional pair-fed control group of sham-operated animals was introduced. Their body weight was 189 ± 10 g (n = 11). There were slight but significant differences in the serum glucose levels among the various groups. Adrenalectomy led to a decrease in serum insulin by 56%, while substitution with cortisol increased the level to 188% of the control value. No differences in the diameter or lipid content of the fat cells was found between the various groups.

*Insulin Binding.* Insulin binding was significantly increased in the adrenalectomised group (Fig. 1). The binding curves for the sham-operated and pair-fed animals were identical, which shows that there was no influence of the reduced food intake on insulin binding. Figure 2 depicts a Scatchard-analysis of the binding data from the different groups. The increase of the receptor capacity of fat cells after adrenalectomy was prevented by cortisol substitution (Fig. 2A). Insulin substitution prevented the increase in the total binding capacity (Fig. 2B), however an upregulation of binding at lower insulin concentrations is still evident.

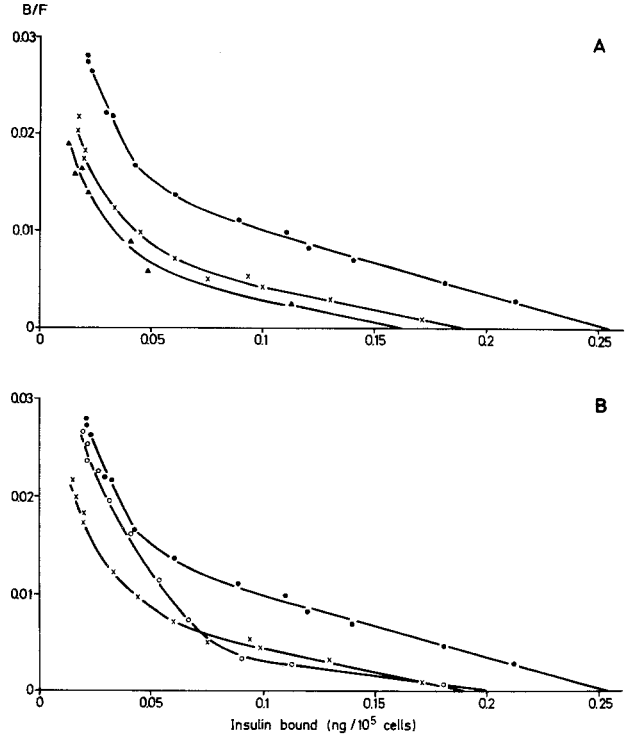


**Fig. 2 A and B.** Scatchard plot analysis of insulin binding. **A** Fat cells of sham-operated (×), adrenalectomised (●) and adrenalectomised, cortisol substituted rats (▲). The points indicate the mean of 8 experiments. **B** The plot of insulin binding to fat cells of adrenalectomised, insulin substituted rats (○) and for comparison the plots of sham-operated (×) and adrenalectomised (●) rats. The points indicate the mean of 8 experiments. The SEM in the adrenalectomised, insulin or cortisol substituted groups ranged from 7.5–14%. For experimental details see Methods section

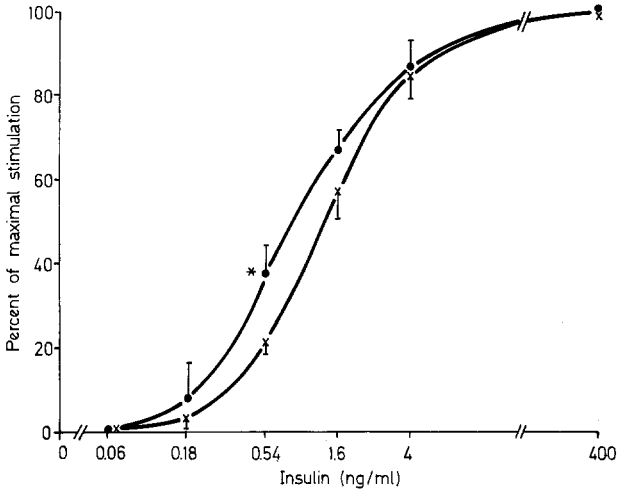


**Fig. 3.** Dose-response curve of the effect of insulin on 3-0-methylglucose transport into fat cells of adrenalectomised (●), and sham-operated (×) rats. The points indicate the mean of 7 experiments ± SEM. Data are expressed as percent of the maximal stimulation. The difference at the insulin concentration 0.54 ng/ml as indicated by the asterix is statistically significant ($p \leq 0.025$). For experimental details see Methods section

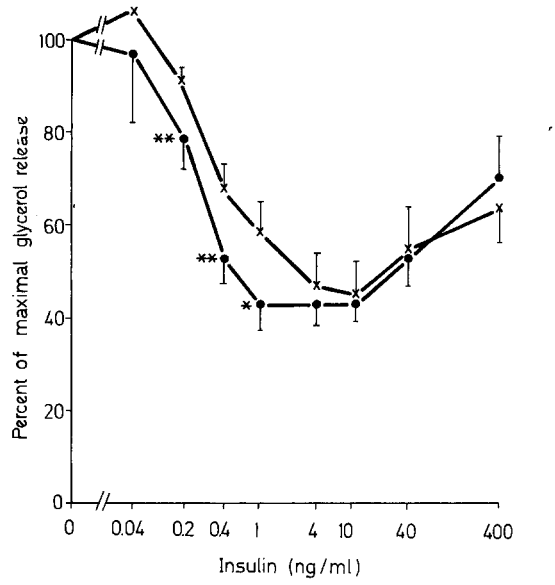


**Fig. 5.** Antilipolytic action of insulin in fat cells of adrenalectomised (●) and sham-operated rats (×). Points indicate the mean of 9 experiments ± SEM and represent the percent inhibition of the glycerol release induced with $10^{-6}$ M isoproterenol. * $p \leq 0.05$, ** p 0.025. For experimental details see Methods section

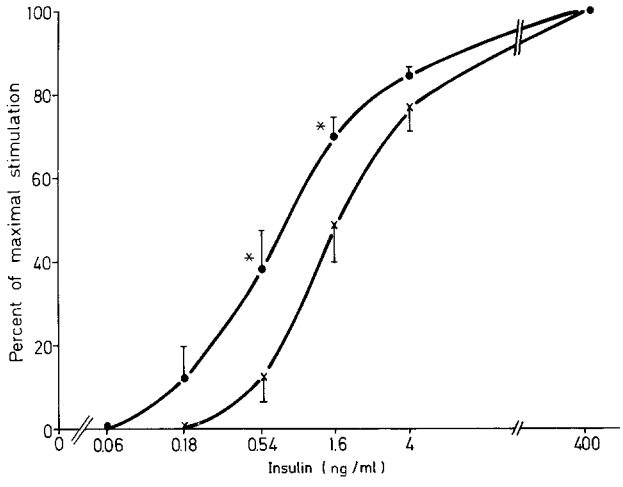


**Fig. 4.** Incorporation of [$^3$H] from glucose into total lipids of fat cells from adrenalectomised (●) and sham-operated rats (×). The points indicate the mean of 6 experiments ± SEM. Data are expressed as percent of maximal stimulation. * $p < 0.025$. For experimental details see Methods section

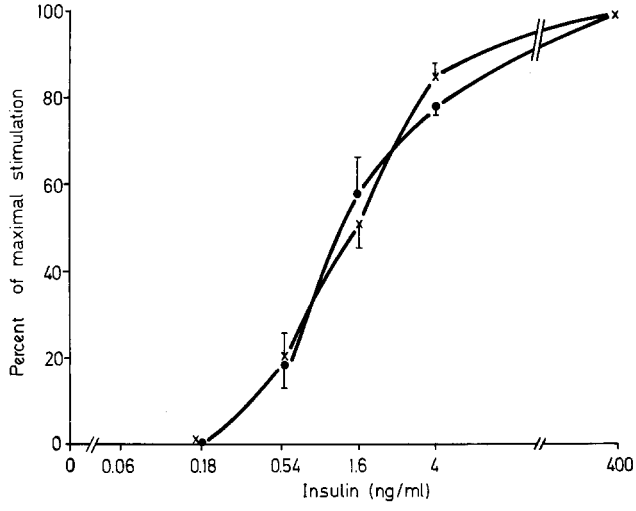


**Fig. 6.** Effect of insulin on the [$^{14}CO_2$]-production from 1-[$^{14}$C]-glucose of fat cells from adrenalectomised rats (●) and sham-operated rats (×). Points indicate the mean of 6 experiments ± SEM. For experimental details see Methods section

*Metabolic Effects of Insulin.* The basal values of 3-0-methylglucose uptake were significantly higher in the adrenalectomised rats (63 ± 4 pmol/$10^5$ cells/15 s) as compared to the controls (35 ± 3 pmol/$10^5$ cells/15 s; $p < 0.01$). The maximal values in the presence of 0.4 μg/ml of insulin were 156 ± 14 in the adrenalectomised, and 128 ± 10 pmol/$10^5$ cells/15 s in the sham-operated rats. The maximal increase of the transport capacity was thus the same in the two groups if it is expressed as the difference of maximal and basal uptake, but was lower in the adrenalectomised groups if it is expressed as ratio of maximal and basal value. Figure 3 shows the insulin effect as percentage of the difference of basal and maximally

stimulated uptake. At submaximal insulin concentrations glucose transport was significantly more stimulated in cells from the adrenalectomised groups. Increased sensitivity of cells from adrenalectomised rats towards submaximal concentrations of insulin was also evident when the incorporation of 2-[$^3$H]-glucose into total lipids was measured (Fig. 4). In this case the basal and the maximally stimulated rates of lipid formation were the same. Another index, the antilipolytic effect of insulin, again revealed an increase in sensitivity to insulin at submaximal concentrations (Fig. 5). As shown before [37], fat cells prepared from the adrenalectomised rats were less sensitive to the effect of the catecholamine. Basal rates of glycerol release were 19.5 ± 3.9 nmol/$10^5$ cells/h in S and 19.9 ± 3.9 nmol/$10^5$ cells/h in A. Isoproterenol ($10^{-6}$ mol/l) stimulated rates of glycerol release were 334 ± 34 nmol/$10^5$ cells/h in S and 269 ± 30 nmol/$10^5$ cells/h in A. In contrast to these dose-response characteristics, no change in the effect of insulin on glucose-1-[$^{14}$C]-oxidation was observed (Fig. 6). The basal rates, 2.5 ± 0.3 nmol/$10^5$ cells/h for control and 2.2 ± 0.3 nmol/$10^5$ cells/h for the adrenalectomised group as well as the maximal rates, 12.2 ± 1.4 nmol/$10^5$ cells/h and 10.8 ± 1.6 nmol/$10^5$ cells/h, respectively, were not different.

## Discussion

The present data indicate, that fat cells from adrenalectomised rats have an increased insulin binding capacity as compared to controls. These results are in contrast to reports of Olefsky et al., who, without showing the data in detail, described no change in the insulin receptors of adrenalectomised rats [38]. Goldfine et al. [39] on the other hand, found indirect evidence for a possible influence of adrenalectomy on insulin binding, showing that the decreased binding induced by the transplantation of an ACTH, growth hormone and prolactin secreting tumour in rats can be reversed by adrenalectomy. In a very recent publication of Kahn et al. [40] an increase of insulin binding in fat cells of adrenalectomised rats was reported, which was solely due to increased affinity. A direct comparison of our binding data with those reported from Kahn et al. is difficult because different tracer concentrations were applied. In the range of insulin concentrations used in our study no change of the affinity was detected, however, such an additional alteration of insulin binding at lower insulin concentrations cannot be excluded. The "up-regulation" of the receptor may be explained by decreased insulin levels in the adrenalectomised rats. Decreased insulin levels after adrenalectomy have been described by several groups [41] and can be explained, at least in part, by reduced responsiveness of the B-cell of adrenalectomised rats to glucose [42]. The inverse relationship between insulin levels and receptor binding has been demonstrated in several experimental models not only in hyperinsulinaemia (for review see [43]), but also in states of insulin deficiency [13–19]. The changes in binding in the adrenalectomised group cannot be explained by their reduced food intake as is evident from the binding data of the sham-operated pair-fed group. This control group seemed to be important, as it is known that fasting induces an increase of insulin binding in rat adipocytes and human monocytes [14, 17, 44, 45].

Cortisol treatment of the adrenalectomised animals prevented the fall in serum insulin and possibly therefore the increase in insulin binding. Though high insulin levels could be achieved a change of the affinity of the receptor still can be observed. Thus cortisol deficiency alone leads to an increase of insulin binding suggesting a direct effect of cortisol on the insulin receptor. Cortisol treatment in vivo leads to a decrease in insulin binding [39, 46]. Since in studies of Olefsky et al. cortisol in vitro had no direct influence on insulin binding in fat cells [47], this was explained as indirect effect mediated by increased insulin levels. The recent finding of Cigolini et al. [48], however, who could demonstrate a direct in vitro effect of cortisol on insulin binding in fat cells supports the above conclusion from our data. An influence of hormones other than insulin on the regulation of the insulin receptor was also demonstrated in experiments with hypophysectomised rats by Schoenle et al. [49].

The increased sensititivity of the fat cells of adrenalectomised rats towards insulin in submaximal doses without a change in the maximal effect can be explained by increased binding capacity of the cells, provided that the concept of "spare receptors" [50, 51] is applied. If the spare receptors are equally able to transmit the insulin signal, an increase of the receptor capacity should not influence the maximal insulin effect, but should lead to a shift of the dose-response curve to the left [52] as is true in our model for glucose transport, lipogenesis and antilipolysis. It is questionable if the lower maximal over basal stimulation ratio of the glucose transport in the cells of the adrenalectomised group can be interpretated as a resistance, as the absolute transport values are higher in the cells of this group than in the cells of the sham-operated group.

Our results confirm earlier findings of Fernandez and Saggerson [25] on the antilipolytic effects of submaximal doses of insulin in adrenalectomised rats. On the other hand, insulin resistance has been

described in these rats, if maximal insulin concentrations are administered and the antilipolytic [23] or lipogenic [23, 25] actions of the hormone are studied, which contrasts with our results. The upregulation of the binding capacity is not reflected in the sensitivity of the glucose oxidation, which indicates changes in the transmission of the insulin signal behind the receptor. The fact, that binding and action are not correlated in all situations was observed in other experimental models by a number of investigators [14, 16, 17, 49, 53, 54].

In addition to the well established phenomenon of an inverse relationship between insulin and receptor levels, our data suggest that cortisol might influence the insulin receptor as well. The hypersensitivity of the target tissue observed in our experiments might contribute to the clinical observation, that adrenal insufficiency in man is associated with the risk of hypoglycaemia and hypersensitivity to the administration of exogenous insulin.

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Dr. H. Häring
Forschergruppe Diabetes
Kölner Platz 1
D-8000 München 40
Federal Republic of Germany