Receptor Binding and Effects of Insulin and NSILA-S on Glucose Transport and Metabolism in Adipocytes from Hypophysectomized Rats

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Summary. Isolated fat cells from normal and hypophysectomized rats have been compared with respect to: 1) binding of insulin and NSILA-S, and 2) effects of these two hormones on glucose transport and metabolism. Although both insulin and NSILA levels were decreased in the serum of hypophysectomized rats, insulin binding was decreased to about 63% of normal, whereas NSILA-S binding remained unchanged. Basal lipogenesis was similar in adipocytes of normal and hypophysectomized rats, but was not stimulated by either insulin or NSILA-S. Similarly, neither of the two hormones stimulated the net gas exchange of "intact" fat pads from hypophysectomized rats. In striking contrast to these findings, 3-O-methylglucose transport in unstimulated fat cells of hypophysectomized rats proceeded at a maximal rate which was not further enhanced by insulin or NSILA-S. These results suggest that the lack of one or several hormones of the pituitary causes one or several enzyme deficiencies responsible for the limited rate of lipogenesis, which otherwiese would proceed at a very rapid rate because of unrestrained glucose transport.

Key words: Insulin, NSILA-S, adipocytes, hypophysectomized rats, insulin-receptor, NSILA-S-receptor, glucose metabolism, glucose transport.

Insulin is an important factor regulating the number of insulin receptors in different types of cells. In adipocytes or liver membranes of hyperinsulinaemic obese hyperglycemic mice (1–5) and in adipocytes and/or monocytes of patients with maturity onset diabetes [6] or obesity [7, 8], both relatively hyperinsulinaemic, the number of insulin receptors is decreased. "Down"-regulation by insulin of the number of insulin receptors was also observed in vitro in IM 9 lymphocytes [9]. In fasted [10, 11] and gentically diabetic [5, 12] animals with clearly decreased serum insulin levels the number of insulin receptors on adipocytes and liver membranes was increased. Increased insulin binding was also demonstrated in fat cells [13] and liver membranes [5, 14] from streptozotocin-diabetic animals and in thymocytes of very young hypophysectomized rats [15].

In contrast, the binding of nonsuppressible insulinlike activity (NSILA-S) to adipocytes of streptozotocin-diabetic rats was decreased [13] despite low concentrations of serum NSILA.

In this study we have tried to answer the following questions:

1) Is the reciprocal relationship between the insulin level and the number of insulin receptors also valid for adipocytes of hypophysectomized rats?

2) Is there an upward regulation of NSILA-S receptors in hypophysectomized rats whose serum NSILA-S-concentrations are even lower than in diabetic rats?

3) Is glucose transport and glucose metabolism in fat cells of hypophysectomized (hypox) rats normal, and are the receptor data reflected in the response of the tissues to these hormones?
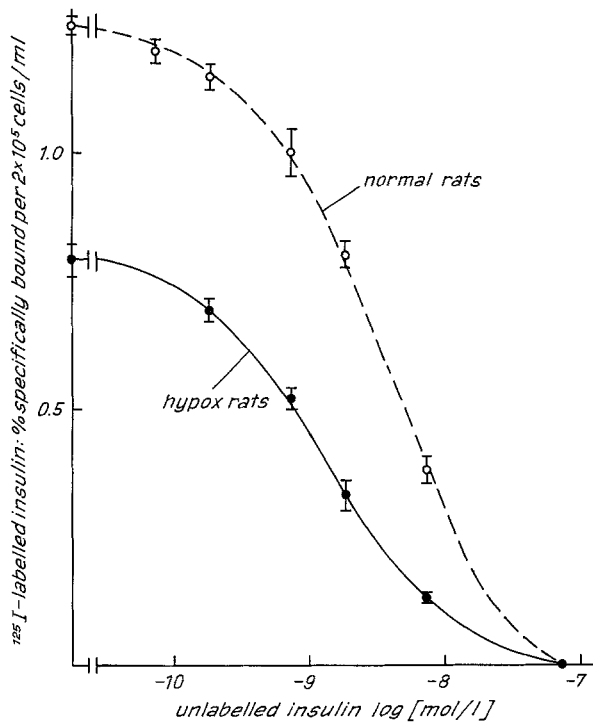
Materials and Methods

Fig. 1. Competitive inhibition of the binding of ^{125}I-labelled insulin by unlabelled insulin in fat cells of normal (O) and hypophysectomized rats (●). Nonspecific binding of ^{125}I-insulin (in presence of 7.5×10^{-8} mol/l of unlabelled insulin) varied between 10–15% of the total binding and was subtracted from all values. Incubation was carried out for 45 min at 37° C. The brackets give the SEM of 5 to 8 experiments carried out in duplicate
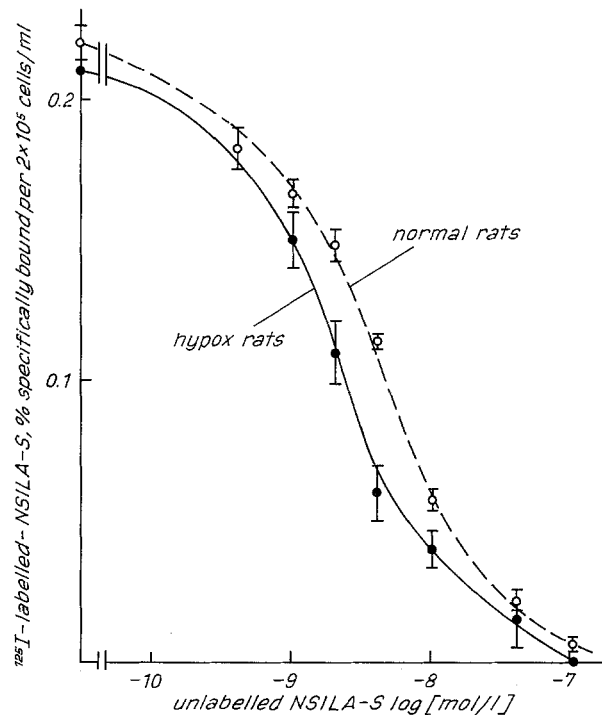
Fig. 2. Competitive inhibition of the binding of ^{125}I-NSILA-S by unlabelled NSILA-S (specific activity 3.8 mU/mg) in fat cells of normal (O) and hypophysectomized rats (●). Incubation lasted for 45 min at 37° C. Nonspecific binding (in presence of 2.0×10^{-7} mol/l of unlablled NSILA-S) varied between 20 and 35% of total binding and was subtracted. Brackets give the SEM of 4 to 8 incubations

Denmark) as standard, anti-porcine insulin serum (guinea pig) and ^{125}I-labelled porcine insulin (CIS, Italy, specific radioactivity 100 μCi/μg). Rat serum was preincubated for 12 h with antiporcine insulin serum at 4° C. Then, ^{125}I-labelled insulin was added for additional 6 h. Bovine γ-globulin (Sigma, 2 mg/ml) was added before precipitation with polyethylene-glycol (according to the prescription of the RIA-gnost insulin kit from Hoechst, Germany). Determination of blood glucose was carried out in a glucose-analyzer (Beckman). Whale insulin (identical amino acid sequence to pork insulin) (24 U/mg) and NSILA-S (3.8 mU/mg) was kindly provided by Drs. E. Rinderknecht and R. E. Humbel. The biological activity of NSILA-S was standardized in the rat fat pad assay with insulin as the reference [17]. The molar concentration of NSILA-S was calculated on the basis of a molecular weight of 7500 [18, 19] and of an activity of ~ 300 mU/mg [20]. Human serum albumin (HSA) was supplied by the Swiss Red Cross, Bern. It was dialyzed extensively against three changes of distilled water and filtered by sterile filtration before use. Streptomycin (5 mg/100 ml) and penicillin (10^6 IU/100 ml) were added.

3-O-Methylglucose was from Sigma, U-^{14}C-3-0-methylglucose and U-^{14}C-glucose from Amersham, Radiochemical Centre. The iodination procedure for insulin and NSILA-S (200 mU/mg, about 65% pure) has been described previously [21].

Experimental Procedures

Fat cells were prepared according the method of Rodbell [22] using crude collagenase (Worthington, Biochemical Centre).

Binding and metabolic studies were carried out in the same manner as described in a preceeding paper [13]. Fat cells (2–4 × 10^5/ml) were incubated for 45 minutes at 37° C in Krebs-Ringer-bicarbonate buffer containing HSA 1 g/100 ml and glucose 1.1 mmol/l. Specific binding of insulin and NSILA-S was measured as detailed in the legends to Figures 1 and 2. Glucose incorporation into total lipids was determined by the method of Gliemann et al. [23, 24].

Transport studies were performed as described earlier [13] with the following slight modifications: The fat cells prepared from fat pads of 8–10 rats were preincubated under gentle stirring for 45 min at 37° C in 3–4 ml of Krebs-Ringer bicarbonate buffer containing 30 mmol/l of U-^{14}C-3-0-methylglucose (~ 20 μCi). The cell suspension was then divided into 4 aliquots. The cells were separated from the buffer by centrifugation through an oil layer (dinonylphthalate, Merck) at 500 g and rapidly resuspended in 5 ml of the same buffer containing cold 3-0-methylglucose (30 mmol/l) alone (control), or, in addition, insulin, (7.5 × 10^{-10} mol/l or 7.5 × 10^{-7} ml/l) or NSILA-S (4.0 × 10^{-8} ml/l). After different time intervals 300 μl aliquots were pipetted into 400 μl microtubes and centrifuged at high speed through a layer (50 μl) of dinonylphthalate [13, 23, 25]. The tops of the microtubes were cut off and counted in 5 ml of Instagel (Packard) in a beta-counter (Tricarb, Packard). The radioactivity recovered in the cell layer after 10 min. in the presence of 7.5 × 10^{-7} mol/l of insulin corresponds to the extracellular water space determined with ^{3}H-insulin (not shown). It was subtracted from all values. The measured radioactivity was extrapolated to zero-time as described earlier [13].
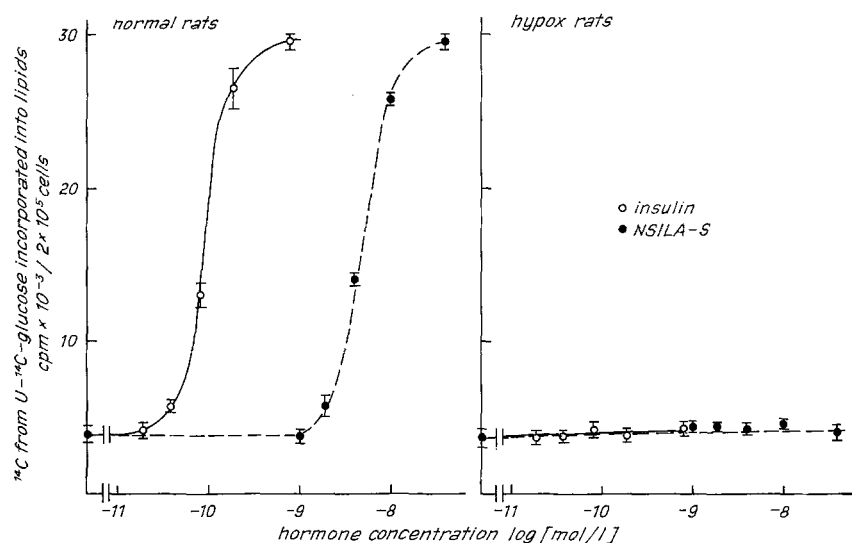
Fig. 3. Lipogenesis from U-^{14}C-glucose in fat cells of normal and hypophysectomized rats stimulated by insulin (four experiments in triplicate) (O) or NSILA-S (one experiment in triplicate) (●). Glucose concentration was 1.1 mmol/l. Incubation was carried out at 37° C for 45 min. The brackets give the SEM

Net gas exchange in whole adipose tissue was measured as described in detail earlier [17].

Results

1) Binding Studies

Figure 1 shows the competitive inhibition of the binding of ^{125}I-labelled insulin by unlabelled insulin in fat cells of normal and hypophysectomized rats.

Specific binding of ^{125}I-labelled insulin in fat cells of hypophysectomized rats was decreased to 63%. Half-maximal displacement occurred at 3.2 × 10^{-9} mol/l in normal adipocytes and at 1.4 × 10^{-9} mol/l in fat cells of hypox rats.

Specific binding of ^{125}I-labelled NSILA-S (Fig. 2) was the same for adipocytes of hypophysectomized and normal rats, 0.22% and 0.21% respectively. Half-maximal "displacement" by unlabelled NSILA-S was achieved at 2.1 × 10^{-9} mol/l in hypox and at 3.5 × 10^{-9} mol/l in normal fat cells.

^{125}I-labelled NSILA-S was not displaced by unlabelled insulin. On the contrary, binding of labelled NSILA-S was increased in the presence of unlabelled insulin. As with adipocytes of streptozotocin-diabetic rats [13] this phenomenon was less pronounced in adipocytes of hypox (+32%) than in adipocytes of normal rat (+180% in the presence of unlabelled insulin, 7.5 × 10^{-9} mol/l; not shown).

2) Metabolic Studies

The differences in the metabolic responses to both insulin and NSILA-S between fat cells of normal and hypophysectomized rats are considerable (Figure 3).

Table 1. Insulin and NSILA-S stimulated net gas exchange in whole adipose tissue of hypophysectomized rats. Experiments were carried out in duplicate, with normal adipose tissue as a control, as described in detail in [17]. The biological activity of NSILA-S was standardized in normal rat adipose tissue with insulin as the reference [17]

	Net gas exchange (μl/g × h)	
	Hypox rats	Normal rats
Insulin: mol/l		
0.75 × 10^{-10}	68 ±12	63 ± 5
2.25 × 10^{-10}	158 ±16	300 ±28
6.75 × 10^{-10}	140 ±31	623 ±31
20.25 × 10^{-10}	150 ±45	845 ±36
NSILA-S: mol/l		
0.4 × 10^{-8}	86 ± 3	
0.8 × 10^{-8}	91 ±12	
1.2 × 10^{-8}	106 ±21	
3.2 × 10^{-8}	108 ± 2	

In normal rats lipogenesis was stimulated by increasing concentrations of insulin (half-maximal at ~ 8.5 × 10^{-11} mol/l) and NSILA-S (half-maximal at ~ 5.1 × 10^{-9} mol/l). In fat cells of hypophysectomized rats (Figure 3) neither insulin nor NSILA-S stimulated lipogenesis to a significant extent.

The basal rate of ^{14}C-incorporation into total lipids was the same in fat cells of normal and hypox rats.

In a control assay, insulin stimulated net gas exchange in intact adipose tissue of hypox rats only to a very small extent compared to adipose tissue of normal rats (Table 1).
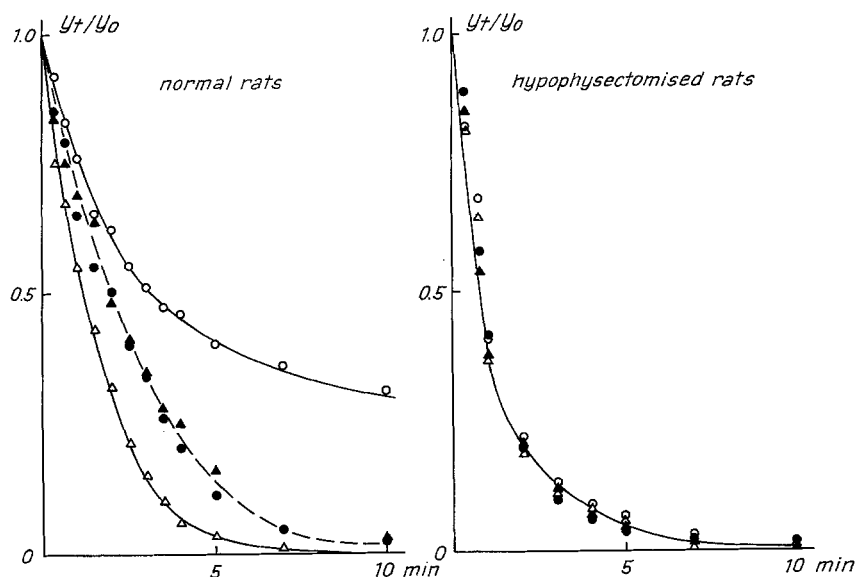
Fig. 4. Efflux of 3-0-methyl-U-^{14}C-glucose from fat cells of normal and hypophysectomized rats. O——O basal efflux; △——△ efflux in the presence of 7.5 × 10^{-7} mol/l of insulin added at zero time; efflux in the presence of insulin (7.5 × 10^{-10} mol/l) ▲——▲ or NSILA-S ●——●, (4.0 × 10^{-8} mol/l) added at zero time. As described in the experimental procedure the value in the presence of 7.5 × 10^{-7} mol/l of insulin obtained after 10 min (between 10 and 20% of the total counts) was subtracted from all values. Each point gives the mean of 3 to 6 incubations. For the sake of clarity the SEM's are not shown. They ranged from 0.01 to 0.04

3) Transport Studies

In Figure 4 the time curves of the efflux of 3-0-methylglucose from adipocytes of normal and hypophysectomized rats are shown. In normal adipocytes the half-life of basal efflux was ~ 180 sec., that of maximally stimulated efflux in the presence of 7.5 × 10^{-7} mol/l of insulin ~ 75 sec. 7.5 × 10^{-10} mol/l of insulin or 4.0 × 10^{-8} mol/l of NSILA-S caused a similar enhancement of 3-0-methylglucose efflux.

Glucose transport in adipocytes of hypophysectomized rats appeared to proceed at a maximal rate already in the basal state. The time curve of the efflux of 3-0-methylglucose in the absence of insulin or NSILA-S was the same as that in the presence of 7.5 × 10^{-7} mol/l of insulin in normal adipocytes. It could not be further stimulated by insulin (7.5 × 10^{-10} mol/l) or NSILA-S (4.0 × 10^{-8} mol/l) nor by a maximal dose of insulin (7.5 × 10^{-7} mol/l) (Fig. 4).

4) Serum Insulin and NSILA-S Levels

Serum insulin levels in hypox rats were (1.5 ±0.1) × 10^{-10} mol/l compared to (3.6 ±0.3) × 10^{-10} mol/l in normal rats (n = 8), whereas the plasma glucose concentrations were in the same range (7.4 ±0.4 for hypox vs. 7.1 ±0.3 mmol/l for normal rats).

The serum NSILA-S levels were (2.4 ±0.8) × 10^{-9} mol/l in hypophysectomized compared to (51.6 ±2.4) × 10^{-9} mol/l in normal rats (n = 6) [26].

Discussion

The results of the present binding studies are in agreement with earlier results obtained in a different strain of rats (Zbz-Cara) [13]: Although specific binding of ^{125}I-labelled NSILA-S to normal adipocytes is somewhat lower than reported previously, half-maximal displacement by unlabelled NSILA-S is similar as is specific binding of ^{125}I-labelled insulin and half-maximal displacement by unlabelled insulin.

At normal blood glucose concentrations the plasma insulin levels are decreased to about 40% of normal in hypophysectomized rats. Nevertheless, we do not find an increase but rather a decrease in insulin binding to isolated fat cells (Fig. 1). This finding is in contrast to the increased insulin binding in thymocytes of hypox rats observed by Goldfine [15] and thus to the current concept that there is a reciprocal relationship between serum insulin levels and the number of insulin binding sites (5–15). Furthermore, NSILA-S binding is unchanged (Fig. 2) in fat cells of hypox rats despite decreased circulating NSILA-S levels.

Lipogenesis in adipocytes of hypophysectomized

rats could not be stimulated by insulin or NSILA-S (Fig. 3). Insensitivity to insulin of adipocytes from hypox rats has also been reported by Eastman and Goodman [27]. We can rule out decreased glucose transport as the cause since basal 3-0-methylglucose efflux in adipocytes of hypox rats is already very fast and cannot be further stimulated by hormones (Fig. 4). Thus, the adipocyte of the hypox rat presents an interesting peculiarity: glucose penetration into the cell no longer seems to be the rate-limiting step of glucose metabolism, and insulin does not appear to play a major role in modulating the availability of glucose to the cell.

In good agreement with the lack of an effect of insulin and NSILA-S on glucose transport is the lack of any detectable effects on glucose metabolism. Since transport is not restricted, the rate-limiting step in glucose metabolism must lie at a more distant level and may involve pyruvate dehydrogenase or fatty acid synthetase [28]. Preliminary results obtained in hypox rats substituted with T3, ACTH and growth hormone, alone and in combination, point to interesting regulatory mechanisms of these hormones in glucose metabolism: Growth hormone treatment restores basal glucose transport and its responsiveness to insulin to normal, whereas treatment with ACTH and T3, at least partly, restores the ability of the fat cell for glucose incorporation into total lipids (Schoenle et al., in preparation).

The possibility that the lack of response of fat cells of hypophysectomized rats to insulin and NSILA-S may be due to cell damage is unlikely: 1) NSILA-S binding is the same in normal fat cells as in fat cells of hypox rats, and the reduction of insulin binding is less drastic than one would expect from the complete lack of any metabolic response to insulin 2) Basal lipogenesis is the same in both conditions. 3) Although very rapid and not responsive to insulin, the basal 3-0-methylglucose efflux follows first order kinetics as does the maximally stimulated efflux in normal fat cells 4) Furthermore, 3-0-methyl-glucose efflux is inhibited by phlorizin (Schoenle et al., in preparation). In agreement with the reported findings in cells, glucose utilization and net gas exchange are barely enhanced by insulin or NSILA-S in intact adipose tissue of hypox rats.

Insulin may regulate the number of its tissue binding sites reciprocally under certain circumstances (1–15). However, our studies show, that this concept cannot be generalized e. g. for hypophysectomized rats: insulin binding to adipocytes is decreased in spite of low serum insulin levels. With respect to NSILA-S binding no consistent correlation with NSILA-S levels in serum is found: Both hypox and diabetic rats have decreased serum NSILA levels.

However, NSILA-S binding is unchanged in fat cells of hypox rats, whereas it is decreased in fat cells of diabetic rats [13].

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