INSULIN AND NONSUPPRESSIBLE INSULIN-LIKE ACTIVITY (NSILA-S) STIMULATE THE SAME GLUCOSE TRANSPORT SYSTEM VIA TWO SEPARATE RECEPTORS IN RAT HEART [1]

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SUMMARY: The effects of NSILA-S, an insulin-like growth factor extracted from human serum, and of insulin on glucose uptake by the perfused rat heart were additive. An additive effect was no longer observed when both hormones were present at maximal concentrations. A maximal transport velocity, determined by the efflux of 3-O-methylglucose, was obtained with each hormone and the effects of the two hormones were not additive. These findings indicated that one single glucose transport system was activated by both hormones. Comparing glucose transport data with receptor binding data, it was concluded that NSILA-S acts via its own receptor on glucose transport in rat heart. Therefore, insulin and NSILA-S appear to stimulate the same glucose transport system via their own specific receptors in rat heart.

INTRODUCTION

Nonsuppressible insulin-like activity (NSILA-S) is an insulin-like growth factor extracted from human serum (1 - 4). Specific receptors for NSILA-S have been shown to be present in chick embryo fibroblasts (5) and chondrocytes (6), rat liver membranes (7), rat fat cells (8) and rat heart muscle (9). The work presented here concerns rat heart muscle. NSILA-S is approximately 2-5 times less potent than insulin in stimulating glucose uptake and efflux of 3-O-methylglucose (10). In heart tissue NSILA-S crossreacts, although with a weak affinity, with the insulin receptor whereas insulin does not occupy the NSILA-S receptor. Therefore, it was of interest to determine whether 1) insulin and NSILA-S stimulate one single or two separate glucose transport systems and 2) whether NSILA-S

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acts on glucose transport via its own specific receptor or via the insulin receptor.

MATERIALS AND METHODS

MATERIALS: Whale insulin (identical with porc insulin) (24 U/mg) and partially purified NSILA-S (3.8 mU/mg) were kindly supplied by Drs. E. Rinderknecht and R.E. Humbel. Human serum albumin was supplied by the Swiss Red Cross. 3-O-methyl-D-glucose was purchased from Sigma, 3-O-methyl-D-(U-^{14}C) glucose from Amersham, Searle.
ANIMALS: The hearts of male, normal fed Zbz-Cara (formerly Osborne-Mendel) rats, weighing between 200 and 230 g were used for all experiments.
EXPERIMENTAL: 1) Glucose uptake: The preparation of rat hearts and the perfusion technique were as described (9,10). Isolated rat hearts were preperfused for 10 minutes with bicarbonate buffer gassed with 95% O_2, 5% CO_2 at 37°C (glucose 10 mmol/l) according to the method of Langendorff (11). After 5 minutes of equilibration in the recirculating perfusion unit the hearts were perfused for another 60 minutes in the presence or absence of hormones (25 ml of bicarbonate buffer, glucose 10 mmol/l, human serum albumin 2 mg/ml). Duplicate samples (0.5 ml) of the perfusion fluid were taken at the beginning and at the end of this 60 minute perfusion period for glucose determination with hexokinase according to Bergmeyer et al. (12). The samples were deproteinized in 3 ml of 0.3 n perchloric acid and neutralized with 6 n potassium carbonate. At the end of the experiment the hearts were frozen with liquid nitrogen (13) and lyophilized overnight for dry weight determination.
2) Transport of 3-O-methylglucose: After 15 minutes of preperfusion, hearts were perfused in a recirculating perfusion system for 45 minutes in order to let the nonmetabolizeable sugar 3-O-methylglucose accumulate intracellularly (3-O-methylglucose: 10 mmol/l, 3-O-methyl-D-(U-^{14}C) glucose: 0.2 µCi/ml, no substrates added). Subsequently, the hearts were rinsed for three minutes with buffer alone to wash 3-O-methylglucose out of blood vessels and interstitial fluid. Efflux measurements were started by changing to a nonrecirculating perfusion unit containing the hormones (basal efflux was measured in the absence of hormones). Fractions of the effluent were collected at intervals of one minute during ten minutes. After that time the hearts were immediately frozen with liquid nitrogen by means of a Wollenberger clamp (13), lyophilized overnight, weighed and extracted with 6% perchloric acid to determine the remaining radioactivity in the heart tissue. The quantity of radioactivity washed out during these 10 minutes plus the quantity remaining in the tissue after that time were taken as the total amount of intracellularly accumulated 3-O-methylglucose (100%). From the specific radioactivity of 3-O-methyl-D-(U-^{14}C) glucose (0.02 µCi/ µmol) the rate of efflux of this substance could be calculated for every minute of the efflux experiment.

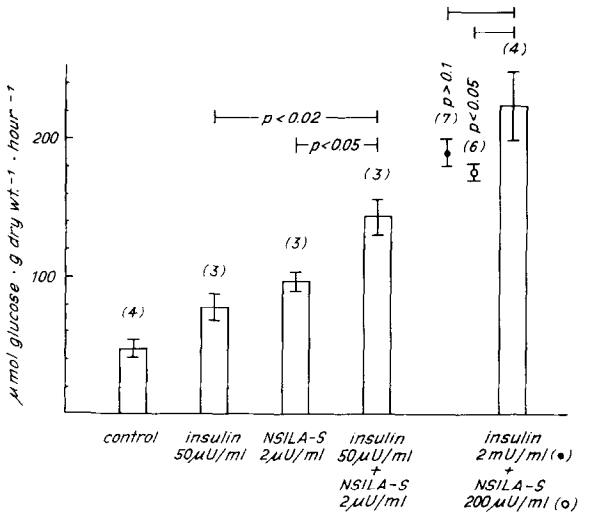
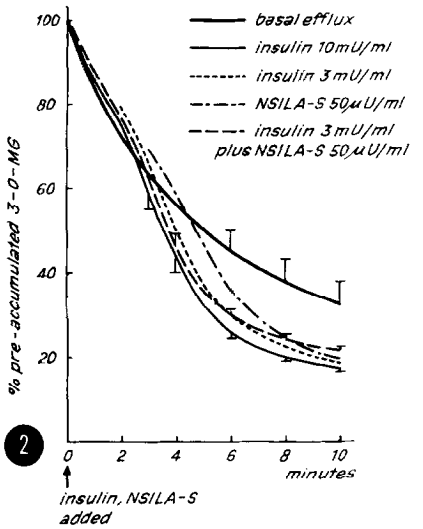


Fig. 1. Glucose uptake by the perfused rat heart. Demonstration of the combined effects of insulin and NSILA-S. Glucose uptake is expressed as µmol glucose consumed per g dry heart weight per hour. Molar concentrations of hormones: insulin 50 µU/ml (3.6×10^{-10} mol/l), NSILA-S 2 µU/ml (7.2×10^{-10} mol/l). The data for insulin 2 mU/ml (1.5×10^{-8} mol/l) (●) and for NSILA-S 200 µU/ml (7.2×10^{-8} mol/l) (o) were taken from (10). Bars indicate the standard error of the mean (SEM), the number of experiments is given in brackets. Student's T-test was used for statistical evaluation.

RESULTS AND DISCUSSION

In order to examine whether insulin and NSILA-S activate only a single or two separate glucose transport systems in rat heart muscle, the combined effects of the two hormones on glucose transport were tested. Figure 1 shows that the effect of the two hormones together at submaximal concentrations was significantly greater than the effect of each hormone alone (insulin 50 µU/ml, NSILA-S 2 µU/ml). In contrast, no additive effect was detectable when both hormones were present at high concentrations at which they exerted maximal effects on glucose uptake (10)(insulin 2 mU/ml, NSILA-S 200 µU/ml) (Fig. 1.). These two findings were indicative of a single glucose transport system activated by both insulin and NSILA-S.



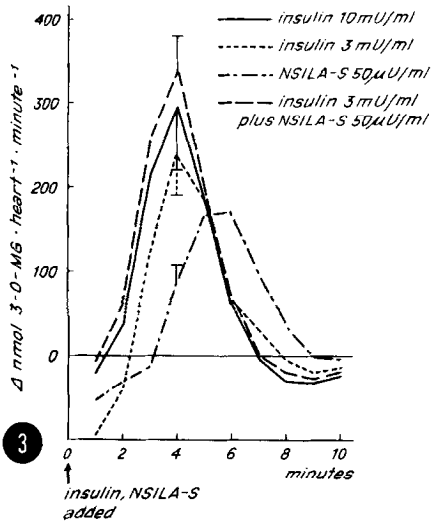


Fig. 2. Time course of 3-O-methyl-D-(U-^{14}C) glucose efflux from perfused rat heart. Combined effect of insulin and NSILA-S. The abscissa gives the time in minutes after changing to a nonrecirculating perfusion unit containing insulin, NSILA-S or both together (basal efflux with no addition of hormones). The ordinate is given as per cent of intracellularly pre-accumulated 3-O-methylglucose. Molar concentrations of hormones: insulin 10 mU/ml (7.3×10^{-8}mol/l), insulin 3 mU/ml (2.2×10^{-8}mol/l), NSILA-S 50 µU/ml (1.8×10^{-8}mol/l). Each curve represents the mean of four separate experiments (NSILA-S 50 µU/ml: 3 experiments). Bars indicate the standard error of the mean (SEM). By using Student's T-test no statistical difference between the curve with insulin 10 mU/ml and the one with insulin and NSILA-S in combination could be detected.

Fig. 3. Efflux rate of 3-O-methyl-D-(U-^{14}C) glucose from perfused rat heart. Combined effect of insulin and NSILA-S. The abscissa gives the time in minutes after changing to a nonrecirculating perfusion unit containing insulin, NSILA-S or both together. The ordinate expresses the difference (Δ) between the hormone stimulated and the basal efflux rate as nmol 3-O-methylglucose washed out per heart during one minute. These data were calculated from the experiments shown in figure 2. Bars indicate the standard error of the mean (SEM). Except for NSILA-S 50 µU/ml at the fourth minute, no statistical differences between the peak rates could be detected.

This hypothesis was further supported by the experiments shown in figure 2 and 3. Here, the efflux of intracellularly pre-accumulated 3-O-methylglucose from perfused rat heart was measured. Insulin (3 mU/ml) and NSILA-S (50 µU/ml) clearly stimulated the efflux. NSILA-S was somewhat less active than

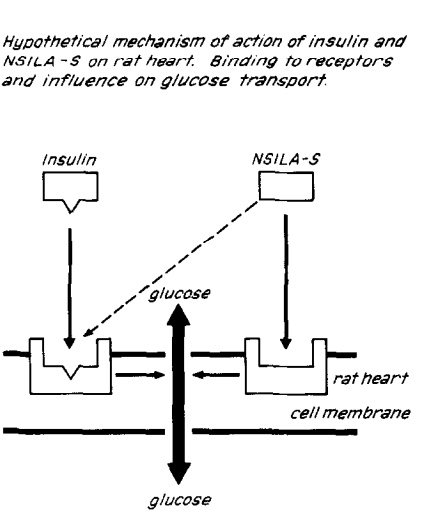


Fig. 4. Schematic summary. Included in this summary are earlier findings from receptor binding studies (9). The hypothesis is put forward that insulin and NSILA-S bind to two separate receptors on rat heart cell membrane. Whereas NSILA-S shows also some affinity for the insulin receptor, the reverse is not the case. Insulin and NSILA-S stimulate the same glucose transport system by interaction with their individual receptors.

insulin. Unfortunately, a higher concentration of NSILA-S could not be tested due to the limited supply of this substance. When insulin (3 mU/ml) and NSILA-S (50 μU/ml) were tested in combination, the stimulation of 3-O-methylglucose efflux was not greater than with a very high dose of insulin alone (10 mU/ml). This was true for the time course of efflux (Fig. 2.) as well as for the efflux rate (Fig. 3.). Therefore, it seems very likely that only a single glucose transport system was involved.

To answer the question whether NSILA-S acts via its own or via the insulin receptor of the rat heart, receptor binding data were compared with data from glucose transport studies. It has been shown that a small quantity of NSILA-S (2 μU/ml, 7.2×10^{-10} mol/l) caused a significant increase of glucose uptake (Fig. 1.). This indicated that the rat heart was very sensitive to small concentrations of NSILA-S. From receptor binding data it was known that about 30 times more NSILA-S than insulin were needed for equal displacement of ^{125}I-labeled insulin from the rat heart (9). This indicated

a poor affinity of NSILA-S for the insulin receptor. Therefore, the hypothesis was put forward that NSILA-S acts via its own receptor on glucose transport in rat heart. The following findings support this concept: Twice as much NSILA-S than insulin was needed to obtain half maximal stimulation of glucose uptake by the perfused rat heart (10). About 5 times more NSILA-S than insulin was needed to obtain similar efflux rates of 3-O-methylglucose (data not shown). The equilibrium constant (K_D) for NSILA-S binding to its specific receptor on rat heart was about 4 times greater than the K_D found for insulin binding (9). The good agreement among these indices supports the conclusion that NSILA-S acts via its own receptor on glucose transport in rat heart.

A schematic summary of all these findings is given in figure 4. It shows that insulin and NSILA-S bind to two separate receptors on the cell membrane of rat heart muscle. In addition, NSILA-S has a low affinity for the insulin receptor. Binding of the hormones to their individual receptors is followed by an increased rate of glucose transport across the cell membrane. The same glucose transport system is activated by both hormones. Our data cannot resolve the question whether or not binding of NSILA-S to the insulin receptor causes any metabolic response. In addition, the exact mechanisms by which these hormones initiate increased glucose transport awaits clarification.

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REFERENCES

1. Oelz, O., Froesch, E.R., Bünzli, H.F., Humbel, R.E. and Ritschard, W.J. (1972) Handbook of Physiology, Vol. 1 (D.F. Steiner and N. Freinkel, eds.) Williams and Wilkins, Baltimore, pp. 685-702.
2. Rinderknecht, E., and Humbel, R.E. (1976) Proc. Natl. Acad. Sci. USA, 73, 2365-2369.
3. Morell, B.,and Froesch, E.R. (1973) Europ. J. clin. Invest, 3, 112-123.

4. Zingg, A.E., and Froesch, E.R. (1973) Diabetologia, 9, 472-476.
5. Zapf, J., Mäder, M., Waldvogel, M., Schalch, D.S., and Froesch, E.R. (1975) Arch. Biochem. Biophys, 168, 630-637.
6. Froesch, E.R., Zapf, J., Audhya, T.K., Ben-Porath, E., Segen, B.J.,and Gibson, K.D. (1976) Proc. Natl. Acad. Sci. USA, 73, 2904-2908.
7. Megyesy, K., Kahn, C.R., Roth, J., Neville, D.M., Nissley, S.P., Humbel, R.E., and Froesch, E.R. (1975) J. Biol. Chem, 250, 8990-8996.
8. Schoenle, E., Zapf, J., and Froesch, E.R. (1976) FEBS-Letters, 67, 175-179.
9. Meuli, C., and Froesch, E.R. (1976) Arch. Biochem. Biophys, 177, 31-38.
10. Meuli, C., and Froesch, E.R. (1975) Europ. J. clin. Invest, 5, 93-99.
11. Langendorff, O. (1895) Pflügers Arch. ges. Physiol, 61, 291-332.
12. Bergmeyer, H.U., Bernt, E., Schmidt, F., and Stork, H. (1970) Methoden der enzymatischen Analyse, Vol. 2 (H.U. Bergmeyer, ed.) Verlag Chemie, Weinheim, pp. 1163-1168.
13. Wollenberger, A., Ristau, O., and Schoffa, G. (1960) Pflügers Arch. ges. Physiol, 270, 399-412.