EFFECT OF INSULIN ON GLUCOSE TRANSPORT AND METABOLISM IN ADIPOSE TISSUE AND SKELETAL MUSCLE OF HYPOPHYSECTOMIZED RATS

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Received 19 October 1981

1. Introduction

Basal glucose transport in fat cells from hypophysectomized (hypox) rats is maximal and insensitive to insulin and basal glucose metabolism in these cells is markedly decreased and not stimulated by insulin [1]. Growth hormone treatment of hypox rats for several days restores glucose transport in the adipocytes towards normal and also restores insulin sensitivity of the glucose carrier system and of glucose metabolism [2]. These findings raised the following question: Are similar alterations encountered in other classicial insulin target organs, such as skeletal muscle, or are they tissue-specific, i.e., characteristic for the adipocyte? To answer this question we first had to reinvestigate glucose transport in whole adipose tissue instead of adipocytes of normal and hypox rats, although strong indirect evidence had been obtained that the above findings are also valid for intact adipose tissue. Here, we show that the alterations in glucose transport and metabolism induced by growth hormone deficiency are specific for adipose tissue and that basal glucose transport and metabolism and their sensitivity to the stimulatory action of insulin are unaffected by hypophysectomy in skeletal muscle.

2. Materials and methods

Hypox male Sprague-Dawley (Tif RAI) rats, (110–140 g body wt) and normal litter mates were generously supplied by Dr Maier and Mr Meier, Ciba Geigy AG (Basel). The animals were fed ad libitum with Altromin chow (no. 1310, totally pathogen-free; Altromin International, Lage) consisting of 23.5% protein, 6% fat and 53% carbohydrates. Hypophysectomy was carried out 3–4 weeks before the experiments were performed. Only those animals were used for the experiments whose body weight did not increase >2 g in 2 weeks.

2.1. *Glucose transport studies in whole adipose tissue*

This method was modified from that in [3]: Epididymal fat pads (75–95 mg) were incubated at 37°C for 1 h in 3 ml Krebs-Ringer bicarbonate (KRB) buffer containing 60 mg human serum albumin and 30 mM 3-*O*-[U-^{14}C]methylglucose (6 μCi). After this preincubation period the pads were rinsed over a piece of nylon stocking with 5 ml KRB–albumin buffer (37°C), rapidly blotted and reimmersed in 3 ml fresh KRB–albumin buffer containing 30 mM of unlabeled 3-*O*-methylglucose. After 10 min incubation (washout period) the tissue was separated from the medium over nylon stocking, blotted on filter paper, and again transferred to 3 ml fresh KRB–albumin buffer containing 30 mM of unlabeled 3-*O*-methylglucose. After 5, 10, 20, 30 and 40 min, 0.1 ml incubation medium was pipetted into 5 ml Instagel (Packard) and counted in a liquid scintillation counter (Packard). At the end of the incubation the tissue was rinsed with 5 ml saline, blotted and homogenized in 1 ml of 1 N NaOH. It was digested by heating for 15 min in a boiling water bath, cooled in ice, neutralized with 1 ml 1 N HCl and centrifuged for 15 min at 1500 × *g*. The fat layer on top was removed with a spatula and 0.5 ml infranatant was added to 5 ml of Instagel and counted.

2.2. *Glucose transport in isolated soleus muscle*

3-*O*-Methylglucose efflux from soleus muscle was measured in a similar 3-step incubation as described for adipose tissue. Soleus muscle (50–60 mg wet wt)

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/S02.75 © 1981 Federation of European Biochemical Societies

were isolated as in [4,5]. Each muscle was attached to a stainless steel holder with a thin nylon thread and placed in a flat-bottomed glass vial (15 ml). It was immersed in 3 ml KRB-buffer, containing 60 mg human serum albumin, 2 mM sodium pyruvate and 10 mM 3-*O*-[U-^{14}C]methylglucose (2 μCi/ml). The preincubation (60 min at 37°C) in a metabolic shaker, gassed with 95% O_2/5% CO_2 (v/v) was followed by a 30 min washout period. The final incubation was carried out in 3 ml fresh KRB–albumin buffer containing 2 mM sodium pyruvate and 10 mM unlabeled 3-*O*-methylglucose for 60 min at 37°C. Aliquots (0.1 ml) of the incubation medium were removed every 10 min, pipetted into 5 ml Instagel and counted in a liquid scintillation counter. At the end of the incubation the muscle was removed from the stainless steel holder, rinsed with cold saline (5 ml), blotted and digested in 0.5 ml 1 N NaOH (by heating for 15 min) and neutralized with 0.5 ml 1 N HCl; 0.5 ml digest was counted in 5 ml Instagel.

2.3. *Glycogen synthesis in soleus muscle from [U-^{14}C]glucose*

The formation of glycogen from [U-^{14}C]glucose was determined as in [4] with slight modifications. The isolated soleus muscles were preincubated for 15 min at 37°C in 2 ml KRB-buffer containing 2 mg glucose and 40 mg human serum albumin in sealed flat-bottomed glass vials in a shaking water bath. During that period they were gassed with O_2/CO_2 (95/5%, v/v). After the preincubation period the muscles were rapidly blotted and transferred on their steel holders to 2 ml fresh KRB-buffer (see above) containing in addition 1 μCi [U-^{14}C]glucose and either 0.3, 1, 3 mU insulin or no hormone (control). The incubation was then continued for 60 min at 37°C in vials sealed with rubber stoppers. They were gassed with O_2/CO_2 during the first 10 min of the second incubation. At the end of the incubation the muscles were rinsed with 5 ml ice-cold saline, blotted on filter paper, removed from the steel holders and digested for 15 min at 90°C in 1 ml 20% KOH containing 5 mg of D^+-glycogen (Fluka); 50 μl of the digest was removed for protein determination following [6]. After cooling in an ice-bath 5 ml absolute alcohol (cooled to −20°C) was added and the samples were kept for 30 min at −20°C. After centrifugation for 10 min at 1500 × *g* the glycogen precipitate was washed twice with 5 ml absolute alcohol to which 100 μl 20% KOH and 100 μl 5% LiBr had been added. After the final centrifugation the precipitate was dissolved in 1.0 ml 0.1 N HCl; 0.5 ml this solution was added to 5 ml Instagel and counted in a liquid scintillation counter.

3. Results and discussion

Fig.1 demonstrates that 3-*O*-methylglucose efflux from isolated fat pads of hypox rats proceeds at a maximal rate and is not further stimulated by insulin. This finding confirms earlier observations in isolated adipocytes [1,2] and extends them to the intact tissue. The lower panel of fig.1 shows that the above finding does not apply to skeletal muscle. The basal

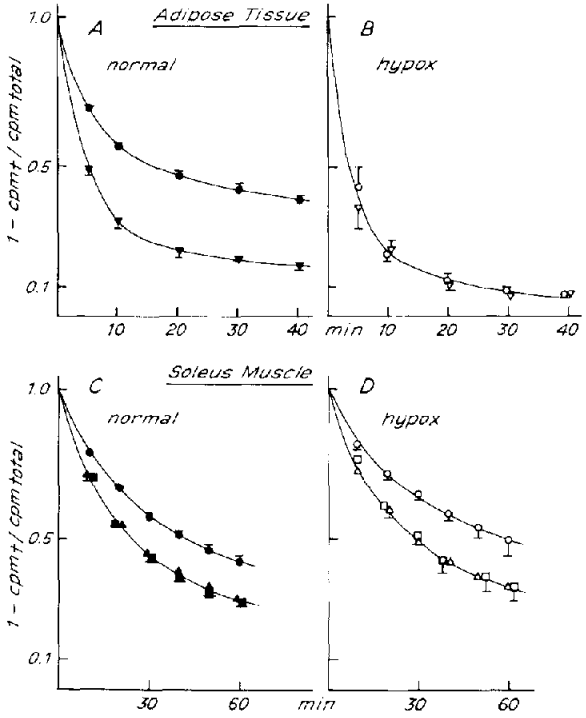

Fig.1. Time course of the efflux of 3-*O*-[U-^{14}C]methylglucose from adipose tissue (A,B) and soleus muscle (C,D) of normal (closed symbols) and hypophysectomized rats (open symbols) in the absence (●,○) and presence of insulin: (■,□) 0.3; (▲,△) 3; or (▼,▽) 10 mU/ml. Incubations were carried out as in section 2. All values are expressed as the ratio of the counts present in the incubation medium at time *t* (cpm_t) and the total counts (cpm_{total}) present in the medium and the tissue at the end of the incubation. This ratio was subtracted from unity (1.0). At every time interval shown this difference reflects the fraction of the total counts released into the medium. Bars give the upper and lower range of 4 incubations from 2 different expt.

3-*O*-methylglucose efflux rate in soleus muscle from hypox rats is not different from that of normal muscle and, like the latter, it is still responsive to insulin. In both normal and hypox rat skeletal muscle, 0.3 mU insulin exert the same stimulatory effect as 3 mU. Thus, the phenomenon that hypophysectomy (and, as shown for adipocytes, growth hormone deficiency in particular) leads to unrestricted glucose transport, is tissue-specific. In contrast to adipose tissue, the glucose carrier system of skeletal muscle does not seem to be under the regulatory influence of the pituitary hormones. In adipocytes, a glucose transport-limiting factor has been postulated [2,7], which restricts glucose transport in the basal state and which is itself inhibited by the action of insulin thus giving rise to increased glucose entry into the fat cell. The limiting cofactor has been shown to be decreased or absent in adipocytes from hypox rats and to be induced by growth hormone treatment of the animals [2]. If, in analogy to adipose tissue, a glucose transport-limiting factor is present in skeletal muscle it must underly a completely different mode of regulation and possess different molecular characteristics.

In adipocytes [1,2] and intact adipose tissue [1] formation of lipids from glucose and glucose oxidation are similar for normal and hypox rats despite the highly increased glucose transport rate in the latter. Insulin barely stimulates glucose utilization in adipocytes or intact adipose tissue of hypox animals [1,2]. In skeletal muscle, basal glycogen synthesis from [U-^{14}C]glucose is similar in normal and hypox rats. In contrast to lipid synthesis in adipose tissue, glycogen synthesis in skeletal muscle is responsive to insulin in the hypox state. It is nearly maximally stimulated by 0.3 mU insulin in both normal and hypox skeletal muscle (fig.2) and the sensitivity to insulin appears to be similar. These data agree with the results obtained for insulin stimulation of glucose transport in fig.1B. Only the glycogen synthesizing capacity is reduced in the hypox condition.

The results obtained for adipocytes [1,2] and for skeletal muscle [this paper] of hypox rats are strikingly similar, although not identical, to those reported for adipocytes and skeletal muscle of hypothyroid rats [8]. In [8] hypothyroidism in adult rats did not significantly change basal glucose utilization in adipocytes, but diminished their response to insulin. In addition, it caused an increase in the basal glucose transport rate. In contrast to adipocytes from hypox rats, however, the transport rates in the presence of

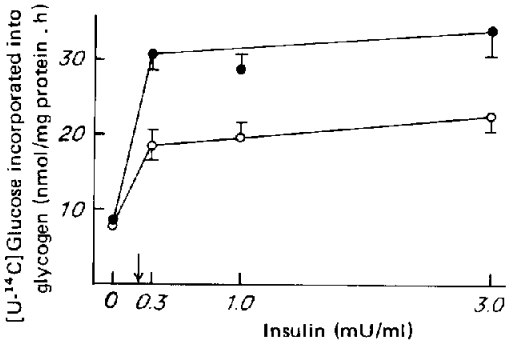

Fig.2. Effect of insulin on the incorporation of [U-^{14}C]glucose into glycogen of soleus muscle from normal (•) and hypophysectomized rats (○). The incubation and glycogen precipitation procedures were as in section 2. Each point is the mean of 4–6 muscles; bars give the SEM. The arrow indicates the approximate insulin concentration where stimulation of glycogen synthesis is half-maximal.

insulin were not significantly different from normal. In skeletal muscle from hypothyroid rats basal and insulin-stimulated glucose transport was unchanged as was basal glucose utilization [8], whereas the responsiveness of glucose utilization to insulin was decreased, although less than in adipocytes of hypothyroid rats [8].

The similarity between the results in [8] and our results is not surprising if one takes into account that the immunoreactive growth hormone level is decreased in hypothyroid rats [9]. In the light of our earlier findings, however, it is growth hormone deficiency per se which is responsible for the drastic alteration of the basal glucose transport rate in adipocytes [2] and for its sensitivity towards further stimulation by insulin [1,2]. The latter phenomenon was not observed in hypothyroidism [8], probably because growth hormone secretion was not sufficiently reduced during the hypothyroid state. Therefore, in contrast to hypothyroidism, growth hormone deficiency achieved by hypophysectomy does not impair the membrane insulin effector system of the adipocyte.

In agreement with [8], in [1,2] the decreased capacity of adipocytes from hypox rats to utilize glucose residues was shown in one or more intracellular enzymes involved in glucose metabolism. T_3 + ACTH treatment of hypox rats restores this capacity in adipocytes towards normal, whereas it does not normalize

glucose transport and its response to insulin [2]. The glucose-transport system in skeletal muscle, however, is not affected by the absence of pituitary hormones (fig.1B). Nor is there a significant alteration in the sensitivity towards insulin of glycogen synthesis (fig.2) (as an index of glucose utilization). The decrease of the maximal response of this index as also observed during hypothyroidism [8] is probably mainly due to the lack of T_3.

The glucose carrier system of adipose tissue responds to growth hormone deficiency in a specific and completely different way from that of the skeletal muscle. Glucose transport in adipose tissue and its sensitivity to insulin are subject to regulation by growth hormone, whereas this is not the case in skeletal muscle.

Acknowledgement

This work was supported by grant no. 3.380-0.78 from the Swiss National Science Foundation.

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