IN VIVO CONTROL OF THE INSULIN-SUPPRESSIBLE HIGH-AFFINITY Ca^{2+}-ATPase OF FAT CELL MEMBRANES BY GROWTH HORMONE AND ITS POSSIBLE INVOLVEMENT IN THE REGULATION OF GLUCOSE TRANSPORT

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1. Introduction

The mechanism by which insulin exerts its acute effects on cell metabolism, such as glucose transport and the low-K_m phosphodiesterase is still unknown. Lately, several interesting observations touching on this problem have been reported (review [1]). One of the discussed mechanisms includes a shifting of calcium within the different cellular pools under the influence of insulin [2–5].

We have investigated insulin effects on glucose transport and metabolism in isolated fat cells of hypophysectomized (hypox) rats [6–8]. We found a maximal glucose transport rate in the basal state and a lack of further effects of insulin. In contrast, glucose incorporation into lipids of these cells was decreased but was also insulin-insensitive [6]. The lipid synthesizing capacity was normalized after treatment of the hypox rats for several days with ACTH together with T_3 [7]. The glucose-transport system was restored after chronic administration of growth hormone (GH) alone to hypox rats [7]: basal glucose-transport rate was decreased to normal and could again be acutely stimulated by insulin. We therefore suggested a GH-dependent 'limiting factor' in the fat cell membrane, which inhibits the glucose-transport system in the basal state. Insulin would release the brakes and enhance the glucose transport [7,8]. Furthermore, we have shown that the activity of insulin-sensitive, low-K_m phosphodiesterase is also increased in fat cells of hypox rats, insensitive to insulin and that growth hormone treatment reverses these alterations so that the same 'limiting membrane factor' may be responsible [9,11].

To learn more about this growth hormone-dependent 'limiting factor' we investigated the low-K_m Ca^{2+}-ATPase in the fat cell membrane of normal, hypox and GH-treated hypox rats. This Ca^{2+}-ATPase in purified fat cell plasma membranes has been characterized [10] and reported to be acutely inhibited by insulin [5]. Here, we show a drastic decrease of the activity and a loss of the insulin-sensitivity of the Ca^{2+}-ATPase after hypophysectomy and their normalization by GH-treatment.

2. Materials and methods

Male Sprague Dawley (Tif RAI) rats (8–16) weighing between 120 and 160 g were used for each experiment. Hypox rats and their normal litter-mates were kindly supplied by Dr Maier and Mr Meier, Ciba Geigy, Basel.

Hypophysectomy was done 3–10 weeks before the experiments. Growth hormone was substituted as in [11]. Human GH (200 mU/day and rat) was injected intraperitoneally for 6 days.

Plasma membranes were purified as in [12]. EDTA was omitted from the fractionation medium [13]. The low-K_m Ca^{2+}-ATPase was assayed as in [5].

Purified plasma membranes (10–30 μg/ml) were incubated for 10 min in Tris–Pipes buffer [12.5 mM, pH 7.4, 37°C containing NaN_3 (20 mM), EGTA (200 μM), $CaCl_2$ (100–200 μM) and [γ-^{32}P]ATP (1 mM)]. The incubation volume was 500 μl. The reaction was stopped by the addition of sodium dodecylsulfate (6 mg/100 μl) and hydrolyzed ^{32}P was extracted and assayed as in [14]. The protein content was determined as in [15]. Free Ca^{2+} was calculated [10] using the affinity constants in [16].

[γ-^{32}P]ATP was from the Radiochemical Centre, Amersham. All chemicals were from Fluka, Buchs. Human growth hormone was a gift from Serono, Rome. Whale insulin (identical amino acid sequence to pork insulin) was kindly provided by Dr R. E. Humbel.

3. Results

A high-affinity Ca^{2+}-dependent ATPase was shown to exist in the fat cell membranes [5]. This enzyme is inhibited by insulin.

The discovery of an insulin-suppressible Ca^{2+}-ATPase in the membrane of fat cells appeared relevant for our model of insulin action on glucose transport and phosphodiesterase activity in hypox and GH-treated hypox rats [6,8], where we had postulated a growth hormone-dependent 'limiting factor' on the fat cell membrane, that itself is acutely inhibited by insulin. The interaction of insulin with this 'limiting factor' would result in an increased activity of the glucose transport [7] and of the low-K_m phosphodiesterase [9,11].

As shown in fig.1, insulin also inhibits the activity of the high affinity Ca^{2+}-ATPase in purified plasma membranes of fat cells of normal rats. Addition of insulin to the membrane preparation inhibits the activity by ~50%. The half-maximal inhibition was achieved at 1.1×10^{-10} M of insulin corresponding rather well to the insulin concentration required for half-maximal stimulation of glucose transport. These results agree well with [5]. In fat-cell membranes from hypox rats we found a marked decrease of the basal activity of Ca^{2+}-ATPase and insulin was without effect. The basal activity was only 40% of that of normal rats. After administration of 200 mU growth hormone/day for 6 days, the basal activity of the Ca^{2+}-ATPase returned towards normal. It was 88% as active as in normal fat cell membranes and became again inhibitory (fig.1).

The dependence of the high-affinity Ca^{2+}-ATPase on the concentration of free Ca^{2+} is shown in fig.2.

The half-saturation constant of the low-K_m Ca^{2+}-ATPase for Ca^{2+} ($K_{0.5}$) was 0.16 μM in adipocyte membranes from normal rats: this agreeing with [5].

The $K_{0.5}$ value was not changed after hypophysectomy. It was 0.15 μM both in hypox and in GH-treated hypox rats.

The V_{max} of the high-affinity Ca^{2+}-ATPase was

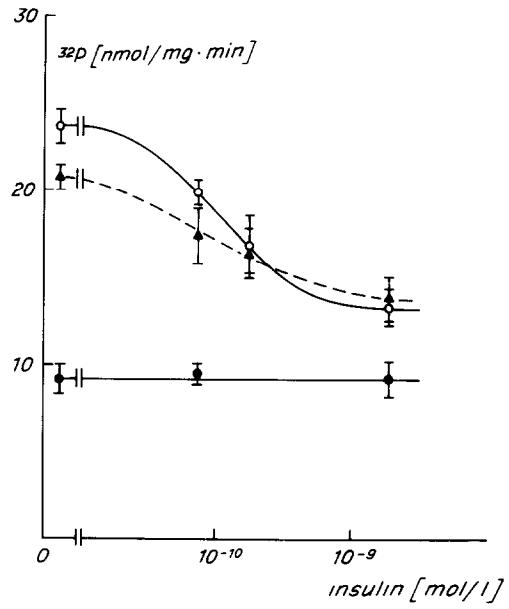

Fig.1. Insulin inhibition of a high affinity Ca^{2+}-ATPase in fat cell membranes of normal, hypophysectomized and growth hormone-treated hypophysectomized rats. Free Ca^{2+} was 47 nM. The Ca^{2+}-ATPase was measured as described in the text. Insulin was added from a human serum albumin (HSA) containing stock solution [7]. The final HSA concentration was 10 mg/l in each sample, also in the absence of insulin. Cell membranes were prepared from (○) normal, (●) hypox and (▲) growth hormone-treated hypox rats. Each point represents the mean ± SEM of 2–6 expt. carried out in duplicates.

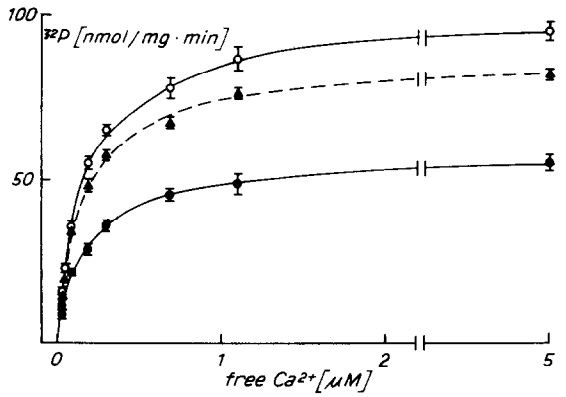

Fig.2. Ca^{2+}-dependence of the high-affinity Ca^{2+}-ATPase in fat cell membranes of normal (○), hypox (●) and GH-treated hypox rats (▲). HSA was omitted. Each point represents the mean ± SEM of the results from different membrane preparations. n = 6 for normal and for hypox rats, and 3 for GH-treated hypox rats.

decreased after hypophysectomy and restored towards normal after GH-administration.

In fat cell membranes of normal rats the V_{max} was 98.3 nmol . mg^{-1} . min^{-1}, in hypox rats 55.7 nmol . mg^{-1} . min^{-1} and in GH-treated hypox rats 85.2 nmol . mg^{-1} . min^{-1}. The decrease of the high-affinity Ca^{2+}-ATPase remains unchanged between 3 and 10 weeks after hypophysectomy.

In contrast to the Ca^{2+}-ATPase, the activity of the 5′-nucleotidase in the plasma membrane fraction remained unchanged for at least 4 weeks after hypophysectomy. The loss of activity which occurred afterwards was not restored after GH-administration (not shown).

It has been suggested [5] that the high-affinity Ca^{2+}-ATPase of isolated fat cells may be an early metabolic step influenced by insulin which regulates intracellular Ca^{2+}-levels. Our data strongly support this contention. They are even compatible with the hypothesis that the high affinity Ca^{2+}-ATPase might represent the proposed growth hormone-dependent 'limiting factor' [7,8] in the fat cell membrane.

It could explain the maximal and insulin-insensitive activities of the glucose-transport system [6] and the low-K_m phosphodiesterase [9,11] in fat cells of hypox rats and their normalization after GH-treatment.

If this Ca^{2+}-ATPase represents a Ca^{2+} extrusion pump in the fat cell membrane, the inhibition of its activity would cause an increase of intracellular Ca^{2+} pools. Increased cytoplasmic calcium may be involved in the regulation of insulin-sensitive metabolic steps, such as the glucose transport through the plasma membrane [2], the low-K_m phosphodiesterase activity, which is not stimulated by insulin in the presence of EGTA [17], and also pyruvate dehydrogenase activity [18].

However, it is unlikely that the inhibition of the GH-dependent high affinity Ca^{2+}-ATPase, by which insulin could control cytoplasmic calcium levels, is responsible for all short-time insulin effects in fat cells. Rather, Ca^{2+} may be involved, possibly as the limiting step in various insulin-sensitive systems, such as the generation of a mediator protein [19–21] or the translocation of a glucose transport system from the cytoplasma to the plasma membrane [22,23]. A more thorough experimental approach to these questions may eventually lead to the understanding of the links between calcium, other cytoplasmic factors and insulin action.

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