Sensitivity of Glucose Uptake and Lipolysis of White Adipocytes of the Rat to Insulin and Effects of some Metabolites

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1. Insulin increased glucose uptake and inhibited lipolysis in white adipocytes of the rat over the same concentration range of the hormone: the half-maximal effects were observed at approx. 10 μ units of insulin/ml. Thus, contrary to previous reports, no difference in sensitivity of the two processes to insulin could be found, which suggests that both these effects of insulin are important in increasing the rate of glucose utilization after a meal. 2. Adenosine deaminase, which lowers the concentration of adenosine in the incubation medium, decreased the sensitivity of both processes (lipolysis and glucose uptake) to insulin: this suggests that adenosine increases the sensitivity of both processes. Similarly, lactate and 3-hydroxybutyrate increased the sensitivity of both processes (to the same extent) to insulin. It is suggested that this increased sensitivity will improve the response (of adipose tissue) to insulin on refeeding after a prolonged period of starvation (when the hydroxybutyrate concentration is high), and after a short burst of exercise, when the blood lactate concentration is high and when large amounts of glucose are produced from lactate via gluconeogenesis in the liver.

It is now well established that fatty acid oxidation in muscle can decrease glucose utilization and oxidation by specific biochemical mechanisms: this effect of fatty acid is part of the glucose/fatty acid cycle (Randle *et al.*, 1963; Newsholme & Randle, 1964; Rennie & Holloszy, 1977). As an extension of this concept, it has been proposed that in normal animals insulin stimulates glucose utilization in muscle primarily via its anti-lipolytic effect on adipose tissue (Newsholme, 1976, 1977). This latter effect lowers the blood concentration of fatty acids, which facilitates glucose utilization and oxidation by muscle. Furthermore, it was suggested that the direct stimulation of glucose utilization by insulin in muscle and adipose tissue (by increasing the rate of membrane transport) requires considerably higher concentrations of insulin. Such values would not be attained in the normal animal except in response to a high-energy high-carbohydrate diet. One obvious biochemical requirement for this hypothesis is that lipolysis in adipose tissue should be more sensitive to insulin than glucose utilization in either adipose tissue or muscle. Although there is some evidence for this in both adipose tissue (see Gries, 1970; Fain *et al.*, 1966) and muscle (Vallance-Owen & Hurlock, 1954), either intact pieces of tissue were used (i.e. adipose tissue from human and rat diaphragm) or, if adipocytes were used, the processes of lipolysis and glucose uptake were not measured under precisely the same conditions. In the present work, the sensitivity of glucose utilization and lipolysis to

insulin were studied with the same adipocytes under the same conditions. We have measured glycerol release as a measure of lipolysis and glucose uptake as a measure of utilization. In addition, the effects of a number of low-molecular-weight metabolites (which may play a role as metabolic messengers) on the sensitivity of these processes to insulin have been investigated. The results are reported and discussed in this paper.

Materials and Methods

Chemicals and enzymes

All chemicals and enzymes were obtained from Boehringer Corp. (London), Lewes, E. Sussex, U.K., except for the following: collagenase (type CLS, batch no. 47E299) was purchased from Worthington Biochemical Corp., Freehold, NJ, U.S.A.; bovine serum albumin (fraction V) was obtained from Sigma (London) Chemical Co., Poole, Dorset BH17 7NH, U.K.; and insulin was a gift from The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, U.K.

Animals

Male Albino Wistar rats were obtained from OLAC (1976) Ltd., Blackthorn, Bicester, Oxon OX6 0TP, U.K., and were kept in the departmental animal house for at least 1 week before use.

Preparation and incubation of adipocytes

Rats, which weighed 100–140 g and had been allowed free access to food and water, were killed by cervical dislocation, and adipocytes were prepared from the epididymal fat-pads by the method of Rodbell (1964). Isolated cells were suspended in Krebs–Ringer bicarbonate buffer (with half the recommended concentration of Ca^{2+}), containing 1 mM-glucose and 4% (w/v) albumin, which had been defatted as described by Chen (1967) and dialysed overnight against 0.9% NaCl. Samples (1 ml) of cell suspension were incubated in plastic scintillation vials in a shaking water bath at 37°C for 1 h. When adenosine deaminase was present in the incubation medium it was present at a final concentration of 10 $\mu\text{g}/\text{ml}$ (2 units/ml); before addition to the medium, the deaminase was dialysed to remove $(\text{NH}_4)_2\text{SO}_4$. Incubations were terminated by the addition of 1 ml of 10% (w/v) trichloroacetic acid. Precipitated protein was removed by centrifugation (1200g for 5 min) and the supernatant was extracted with 3 $\times$ 5 ml of water-saturated diethyl ether to remove the trichloroacetic acid.

Determinations

Glucose concentration was measured by the method of Slein (1965). Glycerol concentration was measured by the method of Garland & Randle (1962). Dry weight was measured on samples (1 ml) of the cell suspension.

Presentation of results

The difference between the initial and final concentration of glucose in the vials is used to measure glucose uptake by the adipocytes, and the results are presented as uptake of glucose. However, it is assumed that such measurements represent glucose utilization (i.e. there is little or no accumulation of intracellular glucose in adipocytes). The results are presented in graphical form to illustrate more readily the responses of the two processes to variations in the insulin concentration. The plots presented are representative of three similar experiments and each point represents the mean of three incubations. The statistical significances of the effects of hydroxybutyrate and lactate have been obtained at each insulin concentration by the use of Student's non-paired *t* test.

Results

The basal rate of lipolysis in adipocytes is very low, so that it is difficult to detect the anti-lipolytic action of insulin. Consequently, in experiments in which the anti-lipolytic action of insulin is to be studied, it is common practice to include a catechol-

amine (e.g. noradrenaline) in the incubation medium to increase the rate of lipolysis. However, catecholamines also increase the rate of glucose uptake by adipocytes (Luzio *et al.*, 1974), which makes it more difficult to detect the effect of insulin on this parameter (although this was not observed in the particular experiment shown in Fig. 2). Since effects of insulin on both parameters were to be investigated in this work, the enzyme adenosine deaminase was used to increase the basal rate of lipolysis in preference to catecholamines. This enzyme converts adenosine into inosine and hence lowers the concentration (in the medium) of adenosine, which is known to be released from adipocytes and is a very potent anti-lipolytic agent (see Schwabe *et al.*, 1975). Furthermore, the presence of this enzyme should ensure a low and steady concentration of adenosine in the medium throughout the incubation period, so that incubation conditions are readily reproducible. Nonetheless, when the effects of adenosine deaminase on insulin sensitivity were tested, noradrenaline was used in place of the deaminase in the incubation medium.

One important finding in this work is that, when adipocytes from the same preparation were used and incubated under the same conditions, changes in the rate of lipolysis and glucose uptake occurred over the same range of insulin concentrations: half-maximal effects were observed at about 10 μ units of insulin/ml for each process (Fig. 1). Since this result was con-

Fig. 1. Effect of insulin on rates of glucose uptake and glycerol release by adipocytes

Adipocytes (32 mg/ml) were incubated in the presence of adenosine deaminase as described in the Materials and Methods section. ●, Glucose uptake; ▲, glycerol release. One unit of insulin is equivalent to 41.7 μg .

trary to expectations and to the reports of other workers (see the introduction), the experiment was repeated with noradrenaline as the lipolytic agent. Changes in the rate of both processes occurred over the same range of insulin concentration, but much lower concentrations were effective (see Fig. 2). Consequently, the effects of adenosine deaminase and noradrenaline on insulin sensitivity were compared. In comparison with the effect of noradrenaline, adenosine deaminase decreased the sensitivity of glucose uptake (as shown by Schwabe *et al.*, 1974) and lipolysis to insulin (Fig. 2). This finding suggests that adenosine increases the sensitivity of both processes to insulin.

It was considered important to investigate whether the sensitivity of glucose uptake and/or lipolysis to insulin could be changed *in vitro* by the presence of other low-molecular-weight compounds. 3-Hydroxybutyrate, acetoacetate, lactate, pyruvate, acetate and butyrate were tested. The sensitivity of both glucose uptake and lipolysis to insulin was markedly increased by 10mM-DL-3-hydroxybutyrate (Fig. 3) or 10mM-lactate (Fig. 4), and the sensitivity of both processes was altered to the same extent. A lower concentration of DL-3-hydroxybutyrate (5mM) had similar but smaller effects (results not shown).

(The effect of the natural isomer, D-3-hydroxybutyrate, has not been investigated.) The effect of 3-hydroxybutyrate on the sensitivity of lipolysis to insulin is somewhat obscured by the fact that 3-hydroxybutyrate inhibits lipolysis in the absence of insulin. Therefore, in Fig. 3(c), lipolytic rates have been expressed as a percentage of the rate observed in the absence of insulin. Although lactate and hydroxybutyrate increased the sensitivity of glucose uptake to insulin, at high insulin concentrations the rate of glucose uptake was decreased by both these compounds. The effects of 10mM-pyruvate, -butyrate, -acetoacetate and -acetate were investigated, but little or no change in sensitivity of either lipolysis or glucose uptake to insulin was detected (results not shown).

Discussion

This study has demonstrated that the processes of lipolysis and glucose uptake in white adipocytes from normal animals have a very similar sensitivity to insulin concentration (the half-maximal effect for both processes is observed at approx. 10 μ units/ml). This finding is not consistent with previous results on the sensitivity of the two processes to insulin. Thus

Fig. 2. Effect of insulin in the presence of noradrenaline or adenosine deaminase on rates of (a) glycerol release and (b) glucose uptake by adipocytes

Adipocytes (21 mg/ml) were incubated as described in the Materials and Methods section. ●, Noradrenaline (1 μ M); ▲, adenosine deaminase (10 μ g/ml).

Fig. 3. Effect of insulin in the presence of DL-hydroxybutyrate on rates of (a) glucose uptake and (b) glycerol release by adipocytes

In (c) rate of glycerol release is presented as a percentage of the rate at zero insulin concentration. Adipocytes (25 mg/ml) were incubated in the presence of adenosine deaminase as described in the Materials and Methods section: ●, no addition; ▲, 10 mM-DL-hydroxybutyrate. The statistical significance (Student's non-paired *t* test) of the difference in the rate of glucose uptake and the percentage rate of glycerol release from the control without hydroxybutyrate is indicated by † ($P < 0.05$), * ($P < 0.01$) and ** ($P < 0.001$).

Fig. 4. Effect of insulin in the presence of lactate on the rates of (a) glycerol release and (b) glucose uptake by adipocytes. Adipocytes (20 mg/ml) were incubated in the presence of adenosine deaminase as described in the Materials and Methods section: ●, no addition; ▲, lithium lactate (10 mM). The statistical significance (Student's non-paired *t* test) of the difference in rates of glucose uptake and glycerol release from the control without lactate is indicated by † ($P < 0.05$), * ($P < 0.02$) and ** ($P < 0.002$).

with pieces of human adipose tissue Gries (1970) observed significant inhibition of lipolysis at 1 μ unit of insulin/ml, whereas significant stimulation of glucose utilization was observed at 50 μ units/ml. Fain *et al.* (1966) have reported that approx. 10 μ units

of insulin/ml just stimulates glucose metabolism, whereas 1 μ unit/ml inhibits lipolysis. It is unclear why there should be such a marked difference. However, since the concentration of compounds such as adenosine (see below) can markedly change the

sensitivity to insulin, we consider that, for comparisons of sensitivity to hormones, it is important that both processes are studied under precisely the same conditions.

The finding that both processes respond to insulin with the same sensitivity suggests that only one insulin receptor is involved in the mediation of effects on both glucose uptake and lipolysis (cf. Newsholme, 1976). If these findings can be extrapolated to the behaviour of adipocytes in the intact animal, they suggest that, in the normal response to the absorption of a carbohydrate meal, insulin increases glucose utilization indirectly by its anti-lipolytic action, together with a direct effect on the rate of glucose uptake by adipose tissue. It is possible that glucose uptake in muscle is less sensitive to insulin than that in adipose tissue. Previous work with the isolated rat hemidiaphragm preparation has suggested that glucose uptake is stimulated by 10–50 μ units of insulin/ml (Vallance-Owen & Hurlock, 1954). However, a problem with the use of whole tissue preparations is that the insulin concentration in the interstitial fluid is likely to be much lower than that in the incubation medium. Furthermore, the sensitivity might be modified by the presence of compounds such as adenosine. We consider that isolated muscle cells are required to test the sensitivity of muscle to insulin. Nonetheless, if muscle tissue is less sensitive to insulin than adipose tissue, then impairment of the sensitivity of adipose tissue to insulin would explain the development of insulin resistance as described by Newsholme (1976, 1977), i.e. failure or impairment of the anti-lipolytic response in adipose tissue would demand that insulin controlled glucose uptake via direct effects on glucose utilization in muscle. Consequently, only high concentrations would be effective.

It has previously been shown that adenosine increases the sensitivity of glucose utilization by adipocytes to insulin (Schwabe *et al.*, 1974; Fernandez & Saggerson, 1978). Since the physiological concentration of adenosine in adipose tissue is not known, it was considered that removal of endogenously produced adenosine by adenosine deaminase is likely to represent physiological changes in the concentration of adenosine. From the effects of adenosine deaminase on the sensitivity of both lipolysis and glucose uptake to insulin (see Fig. 2), it is concluded that physiological concentrations of adenosine can increase the sensitivity of both processes to insulin.

High concentrations of 3-hydroxybutyrate have been shown to inhibit lipolysis in the incubated epididymal fat-pad and adipocytes (Björntorp, 1966; Fain & Shepherd, 1976). This effect has been confirmed in the present work (Fig. 3) and, in addition, DL-hydroxybutyrate or lactate increased the sensitivity of both lipolysis and glucose uptake to

insulin. Madison *et al.* (1964) and Hawkins *et al.* (1971) have shown that infusion of ketone bodies in dogs or rats lowers the plasma non-esterified fatty acid concentration; Houghton (1971) has reported a similar effect after lactate infusion into rats. These changes have been attributed to direct anti-lipolytic effects of the compounds, but the present work suggests an alternative explanation: an increase in the sensitivity of the lipolytic process *in vivo* to insulin. Furthermore, it has been shown that lactate does not inhibit lipolysis in isolated adipocytes (see Fain & Shepherd, 1976, and Fig. 4), but that it does inhibit this process in the incubated epididymal fat-pad. The effects of lactate *in vivo* and in the epididymal fat-pad may both be explained by an increase in the sensitivity to endogenous insulin. (It would be expected that any endogenous insulin would be lost during the preparation of adipocytes.)

The fact that 3-hydroxybutyrate may decrease the rate of lipolysis in at least three separate ways [by a direct antilipolytic effect (see Fig. 3b), by stimulating insulin release from the pancreas (Madison *et al.*, 1964; Jenkins, 1967; Hawkins *et al.*, 1971) and by increasing the sensitivity of the process of lipolysis to insulin (see Fig. 3c)] serves to emphasize the importance of this metabolite as a metabolic regulator of the rate of lipolysis. Furthermore, if it is assumed that the L-isomer of hydroxybutyrate is not active in these experiments, then an increase in insulin sensitivity is seen at 2.5 mM- and 5.0 mM-hydroxybutyrate. The latter concentration is present in the blood of the human subject and the former in the blood of rats, after several days starvation. It is suggested that the importance of this increase in the sensitivity of adipocytes to insulin in the presence of hydroxybutyrate is to ensure effective utilization of absorbed glucose from the bloodstream on refeeding (a doubling of the normal blood glucose concentration approaches that at which loss of glucose occurs through the kidney). Thus, after several days starvation, the elevation of the concentrations of fatty acids and ketone bodies in the blood markedly decreases the rate of glucose utilization by muscle and other tissues (see Newsholme, 1976, for review). On refeeding, the insulin concentration in the blood will increase and, in the initial period after refeeding, the elevated hydroxybutyrate concentration will increase the effectiveness of insulin on glucose utilization and on lipolysis. The effect on the latter process should ensure a rapid decrease in the blood fatty acid and ketone-body concentrations, which will stimulate the utilization of glucose by muscle and other tissues. Indeed, a rapid fall in the blood fatty acid and ketone-body concentrations has been observed in the rat on refeeding of glucose after 48 h starvation (see Newsholme & Start, 1972).

High concentrations of blood lactate are obtained after severe exercise (up to 22 mM; Turrell & Robinson,

1942). Under these conditions, one of the major processes for removal of the lactate is gluconeogenesis in the liver and kidney (see Krebs *et al.*, 1975), so that most of the lactate may be released as glucose into the bloodstream. This could result in a marked increase of the blood glucose concentration. It is important that this glucose is rapidly removed from the blood (see above), which may be encouraged by the effect of lactate on insulin sensitivity in the adipocyte.

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