Hormonal regulation of fructose 2,6-bisphosphate levels in epididymal adipose tissue of rat

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The participation of fructose 2,6-bisphosphate on glycolysis stimulated by insulin and adrenaline in incubated white adipose tissue of rat was investigated. Adrenaline addition to incubated fat-pads strongly decreased the intracellular levels of fructose 2,6-bisphosphate. When the tissue was preincubated with glucose, the presence of insulin in the incubation medium increased fructose 2,6-bisphosphate levels 2-fold. These variations were related to changes in the substrates, ATP and fructose 6-phosphate. It therefore appears that fructose 2,6-bisphosphate may be involved in the control of insulin-induced glycolysis, but it does not seem to play a role in the stimulation of glucolysis by adrenaline.

Fructose 2,6-bisphosphate *White adipose tissue* *Insulin* *Adrenaline* *Glycolysis*

1. INTRODUCTION

While it is well established that adrenaline and insulin increase glycolytic flux and glucose uptake in white adipose tissue [1,2], there are some differences in the concentration of some intermediary metabolites measured after the addition to whole adipose tissue of these hormones. Thus, in the presence of insulin the concentration of glucose 6-phosphate, fructose 6-phosphate and fructose 1,6-bisphosphate increases, and the concentration of ATP and AMP is not altered. By contrast, adrenaline does not modify the concentration of these sugar esters, although ATP levels are decreased [2,3]. Accordingly, it has been suggested that the stimulatory effect of adrenaline on glycolysis operates through PFK-1 activity, since a clear increase in PFK-1 mass-action rate has been observed [2].

On the other hand, it has been shown that Fru-2,6-P_2 is the most potent stimulator of PFK-1 [4]. Also, Fru-2,6-P_2 has been detected in adipose tissue [5]. The properties of PFK-1 from rat adipose tissue extracts are similar to those described in other tissues, in relation to the inhibition by ATP and the activation by cyclic-3′,5′-AMP [6].

This study was undertaken to investigate if the increased glycolytic flux in fat-pads incubated with insulin and adrenaline could be related to the potential changes in Fru-2,6-P_2 levels. The results shown clearly indicate that whereas insulin stimulates Fru-2,6-P_2 synthesis, adrenaline strongly depresses it.

2. MATERIALS AND METHODS

Male Wistar rats (200–250 g) were used in all experiments. Fat-pads were isolated and incubated as in [7], except that 10 mM glucose was added in the preincubation time in some experiments. When refed rats were used, they were starved for 4 days and then refed for several days (experiments shown in table 1) or for 2 days (fig.1). Water was given ad libitum and contained 10% of glucose during refeeding. After 20 min of incubation the fat-pads (100–150 mg) were immediately homogenized in a motor-driven Potter-Elvehjem teflon-glass device with 1 ml of hot 100 mM NaOH and heated for

Abbreviations: Fru-2,6-P_2, fructose 2,6-bisphosphate; PFK-1, phosphofructokinase-1 (EC 2.7.1.11)

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5 min at 80°C. The fat-cake was separated by centrifugation (10000 × g, 5 min) and the neutralized infranatant (at pH 8–8.5) was assayed for Fru-2,6-P_2 as in [8]. For the measurement of other metabolites, the fat-pads were homogenized with 1.5 ml of 0.6 N $HClO_4$. Glucose 6-phosphate, fructose 6-phosphate and ATP were assayed in these neutralized perchlorate extracts as in [9]. Glycogen was measured as in [10]. Glycerol was assayed as in [11] and free fatty acids as in [12] in the incubation medium.

All biochemical and purified enzymes were purchased from Sigma or Boehringer. Insulin (Actrapid) was obtained from Novo Laboratories. Fructose 2,6-bisphosphate was kindly donated by Dr E.V. Schaftingen, Laboratoire de Chimie Physiologique, Université Catholique de Louvain, Belgium.

3. RESULTS

Table 1 shows that Fru-2,6-P_2 is increased in adipose tissue from fasted-refed rats. The values of 0.1 nmol/g tissue found in the fed state agree closely with those previously reported [5]. Since it is well known that the adipose tissue accumulates glycogen during refeeding after a starved period [13], it is possible that the increase in Fru-2,6-P_2 levels is due to its formation from glucose 6-phosphate originating from the spontaneous degradation of glycogen.

Table 1

Effect of alimentary state on Fru-2,6-P_2 levels and glycogen accumulation in adipose tissue

Alimentary state		Fru-2,6-P_2 (nmol/g wet wt)	Glycogen[a] (% wet wt)
Fed	(6)	0.140 ± 0.009	0.001
Fasted (4 days)	(1)	0.062	n.d.[b]
Refed (2 days)	(3)	0.820 ± 0.03	0.56
Refed (3 days)	(3)	1.120 ± 0.01	0.82
Refed (5 days)	(3)	0.620 ± 0.05	0.65

[a] Glycogen values were taken from [10]
[b] n.d., not detectable

In parentheses, the number of observations. Each observation includes pieces of epididymal adipose tissue randomized from 5 rats. The values are means ± SE

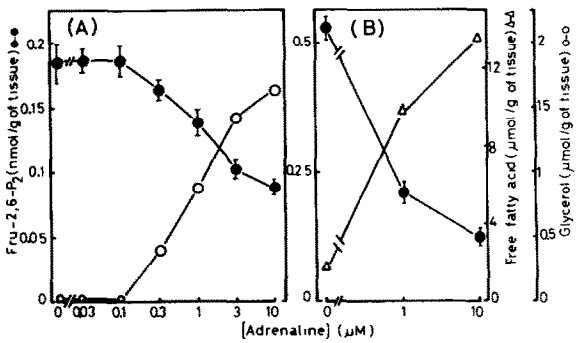

Fig.1. Dose-effect of adrenaline on Fru-2,6-P_2 levels (●) and on the accumulation in the incubation medium of glycerol (○) (A) or free fatty acid (Δ) (B). Adipose tissue from fed (A) or refed (B) rats was used. The fat-pads were preincubated without additions for 20 min and further incubated with 25 mM glucose and adrenaline for 20 min. Values shown are means ± SE (vertical bars) for 3 observations on 3 different preparations of fat-pads.

Fig.1 illustrates that exposure of fat-pads to adrenaline results in a clear decrease of Fru-2,6-P_2 levels. This effect was seen in both fed and refed states. In the first condition (with low glycogen content), the half-maximal inhibition was elicited at 0.5 μM adrenaline. Note the inverse relationship between the decrease of Fru-2,6-P_2 and the rise of glycerol or FFA accumulation in the medium, which suggests that the same molecule(s) regulates both processes, that is, Fru-2,6-P_2 metabolism and lipolysis.

By contrast, insulin has a stimulating effect on Fru-2,6-P_2 synthesis (fig.2). The experiment of this figure is illustrative of a series performed with fat-pads, from fed rats, preincubated with 10 mM glucose before insulin addition. These conditions were selected to study insulin effect, because when the pieces of tissue were preincubated without glucose or if they came from refed rats, irregular data were found. Glucose concentration which elicited maximal response in Fru-2,6-P_2 synthesis was variable between experiments, but with 5 mM glucose clear changes in Fru-2,6-P_2 concentration were already seen. Both insulin and glucose increased Fru-2,6-P_2 levels at 5–10 min after the addition, and remained constant at 20–30 min. A slight decrease was observed at longer time (not shown).

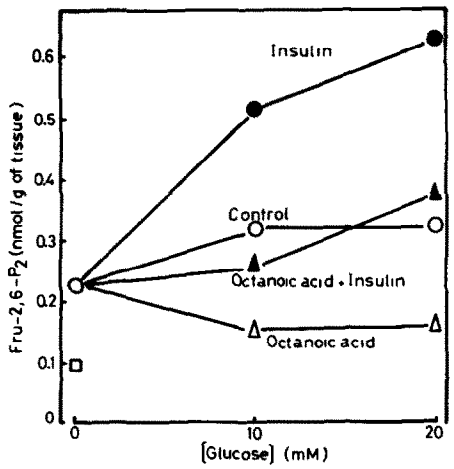

Fig.2. The effect of glucose, insulin and octanoic acid on Fru-2,6-P_2 levels. Fat-pads from fed rats were preincubated with 10 mM glucose for 20 min and further incubated with 10 and 20 mM glucose, 40 mU/ml insulin or 2 mM octanoic acid, as indicated in the figure, for 20 min. Basal Fru-2,6-P_2 content in treated fat-pads without glucose is indicated by (□). Values shown are means for one experiment on 3 different preparations of fat-pads.

Recently, it has been shown that oleic acid stimulates PFK-1 activity in an extract of adipocytes [14]. The effect of octanoic acid on Fru-2,6-P_2 levels in incubated fat-pads is illustrated in fig.2. It was clear that octanoic acid strongly depressed the synthesis of Fru-2,6-P_2 either in the presence of insulin or with glucose alone. Similar results were found with palmitic acid (not shown).

To understand the mechanism responsible for the variations of Fru-2,6-P_2, several metabolites of glycolysis were measured in fat-pads preincubated with 10 mM glucose. The results are presented in table 2. ATP levels were decreased in the presence of adrenaline, but were not modified with insulin. The hexose 6-phosphate content was clearly increased by insulin, but no variations were observed with adrenaline. Octanoic acid decreased insulin-induced ATP levels. These variations are in close agreement with the results previously reported [2,3].

In similar experimental conditions the output of lactate to incubation medium was nearly doubled with insulin (from 1.12 to 2.32 μmol/g tissue). In the presence of adrenaline an increase of about 3-fold was observed.

4. DISCUSSION

The increase by insulin and the decrease by adrenaline of Fru-2,6-P_2 levels in incubated fat-pads described here are in agreement with the suggestion of different mechanisms to explain glycolysis stimulated by both hormones in adipose tissue [2]. The hypothesis has been proposed that the role of Fru-2,6-P_2 in the regulation of liver glycolysis is limited to plethoric conditions, with

Table 2

The effects of adrenaline, insulin and octanoic acid on glucose-6-phosphate, fructose-6-phosphate and ATP in incubated epididymal fat-pads

	Gluc-6-P	Fru-6-P	ATP
	(nmol/g wet wt)		
Control	7.31 ± 0.75	2.52 ± 0.31	165.5 ± 9.1
Adrenaline	5.49 ± 0.49	1.40 ± 0.47	104.0 ± 12.3*[a]
Insulin	17.90 ± 2.05**[a]	5.97 ± 0.86*[a]	173.0 ± 6.2
Insulin + octanoic acid	16.20 ± 1.03	3.05 ± 0.30	112.0 ± 7.9*[b]

[a] vs control
[b] vs insulin

Fat-pads from fed rats were preincubated with 10 mM glucose for 20 min and further incubated with 25 mM glucose and the indicated additions for 20 min. The values are means ± SE for 3 observations, with 5 rats each. * $P < 0.025$, ** $P < 0.01$ (paired differences)

high ATP levels. In these conditions, Fru-2,6-P_2 relieves the ATP inhibition of PFK-1. By contrast, when ATP content is low, the regulatory role is probably not exercised by Fru-2,6-P_2, but other metabolites (AMP, inorganic phosphate, cyclic AMP) play a more important role [15].

The results presented here indicate that this dual control mechanism of glycolysis is also applicable to adipose tissue. Indeed, in the presence of insulin, high levels of ATP and fructose 6-phosphate induce an increase in Fru-2,6-P_2 levels. However, after adrenaline addition the opposite situation was observed. Such a decrease of Fru-2,6-P_2 after adrenaline addition could be correlated with changes in the steady-state whole-tissue concentrations of intermediates likely to be involved in the control of glycolysis by PFK-1 (hexoses phosphate and adenine nucleotides) [2]. In contrast to data presented here no changes in Fru-2,6-P_2 levels were observed after noradrenaline addition to adipocytes [14].

On the other hand, the low levels of Fru-2,6-P_2 found in the presence of octanoic acid could be interpreted by the uncoupler action of fatty acids [16] and by the decrease in tissue concentration of fructose 6-phosphate ([3] and this paper). These data raise the question whether the depressive effect observed on Fru-2,6-P_2 concentration after adrenaline addition is due to an intracellular increase of cyclic AMP, which, in turn, inhibits Fru-2,6-P_2 synthesis [17,18] or this effect is caused by fatty acids produced in the lipolytic process.

The adrenaline effect on Fru-2,6-P_2 levels could also be regulated by other metabolites. Thus, L-glycerol 3-phosphate content is enhanced after adrenaline addition [2]. Since this metabolite stimulates fructose 2,6-bisphosphatase [19], low levels of Fru-2,6-P_2 could therefore be predicted with adrenaline. However, this interpretation does not fit with the fact that insulin increases both L-glycerol 3-phosphate [2] and Fru-2,6-P_2 (this paper).

In summary, whereas the stimulating role on glycolysis exerted by insulin in adipose tissue may be explained by an observed increase in the concentration of Fru-2,6-P_2, the mechanism of the adrenaline effect on glycolysis remains to be proven.

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