Rapid modulation of adipocyte phosphofructokinase activity by noradrenaline and insulin

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1. Alterations in phosphofructokinase properties can be reproducibly seen in tissue extracts prepared and rapidly assayed after exposure of rat adipocytes to hormones. 2. Noradrenaline, corticotropin or isoprenaline (isoproterenol; β -adrenergic agonist) decreased the activity measured with high fructose 6-phosphate concentrations (3–6 mM), but increased activity measured with lower concentrations of this substrate (0.3–0.9 mM). Noradrenaline decreased the $V_{\max}$ and the concentration of fructose 6-phosphate that gave half the $V_{\max}$. 3. Insulin opposed the actions of noradrenaline and itself increased phosphofructokinase activity. 4. The effect of noradrenaline appeared to be exerted through a β rather than an α -type of adrenoceptor. 5. The effects of noradrenaline to decrease phosphofructokinase activity at high [fructose 6-phosphate] and to increase activity at low [fructose 6-phosphate] could be rapidly reversed in cells by addition of the β -blocker propranolol. 6. The effect of noradrenaline seen at low [fructose 6-phosphate] could be abolished by homogenization of cells in buffer containing albumin or reversed by brief incubation of tissue extracts with albumin, suggesting that this effect of the hormone is due to the association of some ligand with the enzyme.

It has recently been shown that rat liver PFK activity is decreased *in vivo* and *in vitro* by glucagon. This effect is seen if the enzyme is assayed with submaximal concentrations of fructose 6-phosphate (Castano *et al.*, 1979; Pilkis *et al.*, 1979; Nieto & Castano, 1980). The effect appears to be associated with phosphorylation of the enzyme (Kagimoto & Uyeda, 1979), parallels inactivation of hepatic pyruvate kinase by glucagon (Castano *et al.*, 1979) and is reported as being either unaffected (Pilkis *et al.*, 1979) or reversed (Castano *et al.*, 1979) by insulin. It has been shown that insulin causes an activation of rat adipose-tissue pyruvate kinase and that adrenaline abolishes this effect (Denton *et al.*, 1979). Accordingly, we have investigated the effect of insulin and noradrenaline on rat adipocyte PFK.

Materials and methods

Chemicals

Fru-6-P, AMP, ATP and coupling enzymes were

Abbreviations used: PFK, phosphofructokinase (EC 2.7.1.11); Fru-6-P, fructose 6-phosphate.

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from Boehringer Corp. (London) (Lewes, Sussex, U.K.). NADH and collagenase (type I from *Clostridium histolyticum*) were obtained from International Enzymes Ltd. (Windsor, Berks., U.K.). L(–)-Isoprenaline (isoproterenol) hydrochloride, DL-propranolol hydrochloride, L-noradrenaline bitartrate and corticotropin (pig) were from Sigma (London) Chemical Co. (Kingston-upon-Thames, Surrey, U.K.). Bovine plasma albumin powder (fraction V) was also from Sigma and was subjected to a defatting procedure (Chen, 1967) with minor modifications (Saggerson, 1972). Bovine glucagon was from Calbiochem (Hereford, U.K.) and phentolamine mesylate from Ciba Laboratories (Horsham, Sussex, U.K.). Before use, coupling enzymes were dialysed overnight at 4°C against 1000 vol. of 50 mM-Tris/HCl buffer, pH 7.4, to remove $(\text{NH}_4)_2\text{SO}_4$.

Animals

These were male Sprague–Dawley rats bred in the animal colony at University College London, and weighed 160–180 g. Animals were fed on GR3EK diet (E. Dixon and Sons, Ware, Herts., U.K.) and had unlimited access to drinking water.

Preparation of adipocytes

Epididymal fat-pads were disaggregated by col-

lagenase (Rodbell, 1964) under the conditions described by Saggerson & Tomassi (1971).

Incubation of adipocytes

Adipocytes equivalent to half of a fat-pad ($7.1 \mu\text{g}$ of DNA) were incubated in 4.0 ml of Krebs-Ringer bicarbonate medium (Krebs & Henseleit, 1932) containing 5 mM-glucose and fatty acid-poor albumin (50 mg/ml). The flask contents were continuously shaken at 37°C and gassed with O_2/CO_2 (19:1). Other additions to incubations were as indicated in individual Tables and Figures.

Analytical methods

After incubation the cells were recovered by centrifugation (15 s at $200g_{av.}$), freeze-stopped in liquid N_2 (Sooranna & Saggerson, 1976) and homogenized in 1.0 ml of 100 mM-Tris/HCl buffer, pH 8.0, containing 100 mM-KF and 5 mM-EDTA with an Ultra-Turrax homogenizer (Sooranna & Saggerson, 1976). The homogenate was centrifuged for 1 min (at $7000g_{av.}$) in an Eppendorf 5412 centrifuge and the resulting infranant below the fat layer taken for assay of enzymes. Immediate assay for PFK was performed spectrophotometrically at 25°C and 340 nm in 1.0 ml containing 33 mM-Tris/HCl buffer, pH 7.4, 200 mM-KCl, 5 mM- MgCl_2 , 0.16 mM-NADH, 1.0 mM-ATP, 0.36 units of aldolase, 15 units of triose phosphate isomerase, 5.1 units of glycerol phosphate dehydrogenase and $40 \mu\text{l}$ of the adipocyte homogenate. Other additions are indicated in Figures and Tables. The reaction, which was initiated by addition of Fru-6-P, was linear for at least 10 min and was proportional to the amount of homogenate added. Lactate dehydrogenase was assayed as described by Saggerson (1974). PFK activity is expressed as nmol of Fru-6-P converted into fructose 1,6-bisphosphate/min per unit of lactate dehydrogenase (PFK/LDH activity ratio). This form of expression corrects for any incomplete cell recovery after incubation and for any incompleteness of cell breakage during homogenization. Glycerol release into incubation media was measured by the method of Garland & Randle (1962). Statistical significance of results was determined by Student's *t* test on a paired basis.

Results

Fig. 1 shows that exposure of adipocytes to noradrenaline or insulin resulted in changes in PFK activity. With insulin, small, but significant, increases in activity were observed at higher Fru-6-P concentrations. With noradrenaline the changes in PFK activity were more complex. At low [Fru-6-P] noradrenaline significantly increased the activity. With 0.3 mM- or 0.6 mM-Fru-6-P severalfold increases in activity were consistently observed. At

Fig. 1. Dependence of PFK activity on Fru-6-P concentration after treatment of adipocytes with noradrenaline or insulin

Adipocytes were incubated for 30 min and then freeze-stopped as described in the Materials and methods section. PFK activity was assayed in the presence of 1.25 mM-AMP. The values are the means for seven adipocyte preparations. The bars indicate S.E.M. and, where not shown, lie within the symbol. * $P < 0.05$, ** $P < 0.025$, *** $P < 0.01$, **** $P < 0.005$ (paired differences). ○, No hormones; ●, with noradrenaline ($0.63 \mu\text{M}$); □, with insulin (1.5 nM); ■, with insulin (1.5 nM) + noradrenaline ($0.63 \mu\text{M}$). The numbers on the Figures indicate the accumulation of glycerol (mM) in the media at the end of the incubations.

high [Fru-6-P] the effect of noradrenaline was the reverse, i.e. a small decrease in PFK activity was observed. Fig. 1(c) shows that insulin and noradrenaline each opposed the action of the other, at both high and low [Fru-6-P]. The data from Fig. 1(a) for the control and noradrenaline-treated cases were fitted to the expression

$$v = \frac{V_{\max} [S]^n}{K + [S]^n}$$

where n and K are variables, by using the computer program FACSIMILE (Chance *et al.*, 1977). The optimization routine finds the optimum values of $V_{\max}$, n and K that minimize the normalized residual sum of squares. Chi-squared per degree of freedom was 1.33 and 1.14 in the control and noradrenaline-treated cases respectively, indicating a satisfactory fit of the data. In the control case the values obtained ($\pm$ s.d.) were $V_{\max} = 27.2 \pm 1.0$, $K = 1.73 \pm 0.13$, $n = 1.82 \pm 0.09$. On incubation with noradrenaline, the values were $V_{\max} = 17.6 \pm 0.3$, $K = 0.33 \pm 0.03$ and $n = 1.64 \pm 0.08$. Thus treatment of the cells with the hormone decreased $V_{\max}$ (under the chosen assay conditions by 35% and $K_{0.5}$ (the Fru-6-P concentration giving 50% of $V_{\max}$) by 60%. It is noteworthy that there are several reports of catecholamine-mediated increases of PFK activity at low [Fru-6-P] in skeletal muscle, heart or diaphragm (Mansour, 1972; Nakatsu & Mansour, 1973; Beitner *et al.*, 1978; Clark & Patten, 1981). In other experiments (results not shown), the effects of insulin and noradrenaline respectively to increase and to decrease PFK activity at high [Fru-6-P] (3 mM) were also seen when AMP was replaced by 0.1 mM-cyclic AMP or when AMP (1.25 mM) + citrate (4 mM) were present. In the absence of an

adenine nucleotide activator the hormonal effects were not apparent. Table 1 shows that corticotropin and the selective β -adrenoceptor agonist isoprenaline had similar effects to noradrenaline at both high and low [Fru-6-P]. Glucagon, however, was ineffective, although there was a small stimulation of lipolysis by this agent. Involvement of a β - rather than an α -type of adrenoceptor in the effect of noradrenaline at high [Fru-6-P] is implied by the fact that the β -blocker propranolol abolished the effect of noradrenaline, whereas the α -blocker phentolamine was ineffective (Table 1). The noradrenaline-induced increase in PFK activity at low [Fru-6-P] was also blocked by propranolol (see Fig. 3 below). In the experiments described above, freeze-stopped adipocytes were homogenized in the Tris/KF/EDTA buffer described in the Materials and methods section. The two effects of noradrenaline seen when this homogenization buffer is used can be separated, since inclusion of albumin in the homogenization buffer slightly enhanced the effect of the hormone at high [Fru-6-P] (6 mM), but greatly diminished the effect seen at 0.6 mM-Fru-6-P (Table 2). Fig. 2 shows the Fru-6-P-dependence of PFK in albumin-containing extracts, and again, by comparison with Fig. 1(a), it may be seen that the effect of noradrenaline under this condition is almost confined to the decrease in activity at high [Fru-6-P]. In this respect it is noteworthy that the rapid inactivation of adipocyte phosphatidate phosphohydrolase by noradrenaline (Cheng & Saggerson, 1980) is also abolished when albumin is included in the homogenization buffer. For this latter enzyme the hormone effect could not be reversed if albumin was added after the preparation of the homogenate (Cheng & Saggerson, 1980). However, this was not found with PFK. Table 3 shows that the increase in

Table 1. *Effects of lipolytic agents, phentolamine and propranolol on PFK activity*

Adipocytes were incubated for 30 min and freeze-stopped and homogenized as described in the Materials and methods section. PFK activity was assayed in the presence of 1.25 mM-AMP. The values are means $\pm$ s.e.m. for four adipocyte preparations * $P < 0.025$, ** $P < 0.01$, *** $P < 0.005$ for hormone-treated cells versus appropriate controls (paired differences).

Additions to adipocyte incubation	PFK/LDH activity ratio			Glycerol accumulation in incubation media (mM)
	With 0.6 mM-Fru-6-P	With 0.9 mM-Fru-6-P	With 6 mM-Fru-6-P	
None	0.9 $\pm$ 0.1	1.6 $\pm$ 0.2	15.2 $\pm$ 1.0	0.02 $\pm$ 0.0
Isoprenaline (2.5 μ M)	2.3 $\pm$ 0.3***	4.2 $\pm$ 0.2***	10.4 $\pm$ 0.3**	1.28 $\pm$ 0.05***
Corticotropin (100 ng/ml)	2.1 $\pm$ 0.3***	3.8 $\pm$ 0.5***	10.7 $\pm$ 0.5**	1.07 $\pm$ 0.16***
Noradrenaline (0.63 μ M)	2.1 $\pm$ 0.1***	4.8 $\pm$ 0.3***	9.9 $\pm$ 0.4**	1.20 $\pm$ 0.04***
Glucagon (100 ng/ml)	—	—	14.9 $\pm$ 0.8	0.31 $\pm$ 0.10
Phentolamine (5 μ M)	—	—	14.2 $\pm$ 0.7	0.05 $\pm$ 0.01
Phentolamine (5 μ M) + noradrenaline (0.63 μ M)	—	—	10.5 $\pm$ 0.4*	1.11 $\pm$ 0.14***
Propranolol (5 μ M)	—	—	15.3 $\pm$ 0.4	0.05 $\pm$ 0.01
Propranolol (5 μ M) + noradrenaline (0.63 μ M)	—	—	14.7 $\pm$ 0.9	0.08 $\pm$ 0.03

Fig. 2. Dependence of PFK activity on Fru-6-P concentration in adipocyte extracts prepared in the presence of albumin

Adipocytes were incubated for 30 min, freeze-stopped and homogenized as described in the Materials and methods section, except that the homogenization buffer contained fatty acid-poor albumin (20 mg/ml). PFK activity was assayed in the presence of 1.25 mM-AMP. The values are the means for four adipocyte preparations and the bars indicate S.E.M. **P* < 0.05, ***P* < 0.025, ****P* < 0.005 (paired differences). ○, No hormones; ●, with noradrenaline (0.63 μM).

PFK activity at low [Fru-6-P] seen with noradrenaline can also be swiftly reversed by incubation with albumin added after homogenization. It is concluded that the effect of noradrenaline to increase PFK activity at low [Fru-6-P] is due to the interaction of some ligand with PFK and that this ligand can be removed and bound by albumin. If such a ligand is a lipolysis product or is derived from the products of lipolysis its nature is unclear. Incubation of extracts from control cells with 1 mM-sodium palmitate or with 200 μM-palmitoyl CoA (5 min at 25°C) did not mimic the effect of noradrenaline (results not shown). Table 3 also shows that mixing and incubation of equivalent amounts of extracts from noradrenaline-treated and control cells resulted in measured PFK activity appreciably less than the mean of the two individual activities. Fig. 3 demonstrates the rapid reversal within cells of noradrenaline-induced changes in PFK activity. In this experiment sufficient propranolol was added to block noradrenaline action, as evidenced by the complete arrest of lipolysis (Fig. 3a). Figs. 3(b) and 3(c) show that this β-adrenoceptor blockade rapidly abolished the effects of noradrenaline on the enzyme. Fig. 3(c) also shows that PFK activity with 6 mM-Fru-6-P increased on commencement of incubation of the cells in the

Table 2. Effect of homogenization in albumin-containing buffer on noradrenaline-induced changes in PFK activity
Adipocytes were incubated for 30 min and freeze-stopped as described in the Materials and methods section. PFK activity was assayed in the presence of 1.25 mM-AMP. The values are means ± S.E.M. for four adipocyte preparations. The values in parentheses indicate the percentage changes caused by noradrenaline. **P* < 0.05, ***P* < 0.005 respectively for effects of noradrenaline (paired differences).

Noradrenaline (0.63 μM) in cell incubation	Albumin (20 mg/ml) in homogenization buffer	PFK/LDH activity ratio	
		With 0.6 mM-Fru-6-P	With 6 mM-Fru-6-P
—	—	1.0 ± 0.2	16.8 ± 0.8
+	—	6.7 ± 1.1** (+545 ± 25%)	11.6 ± 0.8** (−31 ± 3%)
—	+	0.8 ± 0.3	15.9 ± 2.3
+	+	1.1 ± 0.4* (+47 ± 18%)	9.6 ± 1.9** (−41 ± 6%)

Table 3. Effect of incubation in albumin-containing buffer on noradrenaline-induced changes in PFK activity
Adipocytes were incubated for 30 min with or without 0.63 μM-noradrenaline, freeze-stopped and homogenized in albumin-free buffer as described in the Materials and methods section. Extracts were either assayed directly or diluted 1:1 and incubated at 25°C for 5 min before assay. The diluting buffer was either albumin-free homogenization buffer or the same containing fatty acid-poor albumin (40 mg/ml). PFK was assayed in the presence of 1.25 mM-AMP. The values are means ± S.E.M. for three adipocyte preparations.

Origin of cell extract	PFK/LDH activity ratio (with 0.6 mM-Fru-6-P)		
	Non-incubated extracts	Extracts incubated in albumin-free buffer	Extracts incubated in buffer with albumin
Control cells	0.7 ± 0.1	0.8 ± 0.1	0.9 ± 0.1
Noradrenaline-treated cells	3.1 ± 0.1	4.2 ± 0.5	0.4 ± 0.1
Control + noradrenaline-treated cells (1:1)	—	1.5 ± 0.2	—

Fig. 3. Rapid reversal by propranolol of effects of noradrenaline on PFK activity

Adipocytes were incubated as described in the Materials and methods section and freeze-stopped at the indicated times; 5 μM-DL-propranolol was added to some flasks at 10 min. PFK activity was assayed in the presence of 1.25 mM-AMP. * $P < 0.025$, ** $P < 0.01$, *** $P < 0.005$ for effects of noradrenaline (paired differences). ○, No hormone; ●, with

glucose-containing incubation medium and that noradrenaline appeared to prevent this increase rather than itself to lower the activity. Noradrenaline appeared to block the production or action of some activating factor.

Discussion

These findings suggest that adipocyte PFK is subject to rapid, reversible alterations of kinetic properties by hormones. The overall effect of noradrenaline is quite different from the effect of glucagon on hepatic PFK, since noradrenaline decreases rather than increases the $K_{0.5}$ for Fru-6-P and decreases the V_{max} , which in the case of the liver enzyme is unchanged (Castano *et al.*, 1979; Pilakis *et al.*, 1979; Nieto & Castano, 1980). The present findings also differ from those with the hepatic enzyme (Castano *et al.*, 1979; Pilakis *et al.*, 1979) in that insulin appears to have a small effect in its own right. Whole adipose tissue contains at least two chromatographically distinct forms of PFK (Taylor & Bew, 1970), possibly corresponding to skeletal-muscle and liver isoenzymes. Chromatography of rat adipocyte post-105000g supernatants on DEAE-cellulose results in a similar resolution of PFK into two distinct peaks (S. J. Smith & E. D. Saggerson, unpublished work). Observed changes in PFK activity in the present experiments could therefore represent a complex summation of effects on one or on both forms of the enzyme. Furthermore, at least two effects of noradrenaline are apparent. Although persisting through freeze-stopping and homogenization, the increase in activity at low [Fru-6-P] appears to involve the reversible binding of some ligand. Although removal of this putative ligand with albumin diminishes the effect at low [Fru-6-P], the decrease in activity at high [Fru-6-P] is unaffected by this treatment. It is not established whether the effect at high [Fru-6-P] is due to binding of effector molecules or could be due to covalent modification of the enzyme. The necessity for relatively high Fru-6-P concentrations to assay the enzyme in fresh extracts of adipocytes almost certainly does not reflect the behaviour of the

noradrenaline (0.63 μM); □, with propranolol (5 μM); ■, with noradrenaline (0.63 μM) + propranolol (5 μM). (a) Lipolysis (glycerol accumulation in incubation media). Values are means of six adipocyte preparations. (b) PFK assayed in albumin-free homogenates at 0.6 mM-Fru-6-P. Values are means of three adipocyte preparations. (c) PFK assayed at 6 mM-Fru-6-P after homogenization of adipocytes in buffer containing fatty acid-poor albumin (20 mg/ml). Values are means of three adipocyte preparations.

enzyme in the cell. Because of the large fat content of the cells, fresh extracts are, of necessity, very dilute. As a result, abnormally high Fru-6-P concentrations may be required, either because of the low protein concentrations used (Reinhart & Hardy, 1980) or because of dilution and removal of activating factors (Uyeda *et al.*, 1981). Flux through adipocyte PFK provides both carbon for the glycerol moiety of triacylglycerols and carbon destined for synthesis of fatty acids *de novo*. The rates of formation of these two products are not necessarily proportional to each other, because the adipocyte can also synthesize triacylglycerols from fatty acids released by lipolysis or obtained from plasma lipoprotein triacylglycerols. Both adrenaline and insulin appear to increase glycolytic flux and hence PFK activity in incubated adipose tissues (Halperin & Denton, 1968; Saggerson & Greenbaum, 1970). In the case of insulin this could be achieved by the observed rise in the tissue content of Fru-6-P (Halperin & Denton, 1968; Saggerson & Greenbaum, 1970). However, with adrenaline the increase in PFK flux is achieved without an increase in Fru-6-P (Halperin & Denton, 1968; Saggerson & Greenbaum, 1970), and the action of other effectors must be invoked. Further studies should be directed towards clarification of the nature of the two noradrenaline-directed effects seen here and also towards investigation of the role in adipose tissue of 'activation factors' such as fructose 2,6-bisphosphate, which have recently been shown to be important in the hormonal regulation of hepatic PFK (Furuya & Uyeda, 1980; Van Schaftingen *et al.*, 1980; Richards & Uyeda, 1980; Pilkis *et al.*, 1981; Uyeda *et al.*, 1981).

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