Biochem. J. (1987) 241, 415–419 (Printed in Great Britain)

Catecholamine activation of pyruvate dehydrogenase in white adipose tissue of the rat *in vivo*

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Intraperitoneal injections of noradrenaline or adrenaline into rats increased the proportion of pyruvate dehydrogenase in the active state in white adipose tissue; this effect of catecholamines was also apparent in streptozotocin-diabetic rats, showing that it was not due to an increase in serum insulin concentration. The catecholamine-induced increase in pyruvate dehydrogenase of white adipose tissue *in vivo* was completely blocked by prior injection of either the β-antagonist propranolol or the α_1-antagonist prazosin. Cervical dislocation of conscious rats increased pyruvate dehydrogenase activity of white adipose tissue, which was prevented by prior injection of propranolol. Adrenaline (30 nM) activated pyruvate dehydrogenase in white adipocytes *in vitro*; the maximum effect of adrenaline required activation of both α_1- and β-receptors. The results show that catecholamines activate pyruvate dehydrogenase of white adipose tissue both *in vivo* and *in vitro* and that this effect is mediated by a combination of α_1- and β-adrenergic receptors.

INTRODUCTION

Pyruvate dehydrogenase (PDH) has a major role in the regulation of glucose metabolism to acetyl-CoA for oxidation to CO_2 or for fatty acid synthesis in white adipose tissue. The enzyme occurs in an active, dephosphorylated, form and an inactive, phosphorylated, form, the interconversions being effected by PDH kinase and phosphatase (see Saggerson, 1985). Insulin increases the amount of PDH in the active state in white adipose tissue both *in vivo* and *in vitro* by stimulation of the phosphatase; this effect is an important component of the mechanism whereby insulin stimulates glucose oxidation and fatty acid synthesis (see Saggerson, 1985).

Whereas insulin promotes glucose utilization and fat synthesis in adipose tissue, catecholamines promote lipolysis both *in vivo* and *in vitro* (see Vernon & Clegg, 1985). Catecholamines also inhibit acetyl-CoA carboxylase activity *in vivo* (Lee & Kim, 1979) and *in vitro* (see Saggerson, 1985). In contrast, catecholamines stimulate glycolysis in white adipose tissue *in vitro* (see Saggerson, 1985), in part at least through activation of phosphofructokinase (Sale & Denton, 1985). The effects of catecholamines on PDH of white adipose tissue, however, are unclear, for they have been reported to inhibit (Coore *et al.*, 1971), activate (Taylor *et al.*, 1973; Weiss *et al.*, 1974) or have biphasic effects on (Sica & Cuatrecasas, 1973; Smith & Saggerson, 1978; Cheng & Larner, 1985) the enzyme. As all of these studies on PDH have been performed *in vitro*, we have investigated the effects of catecholamines on PDH of white adipose tissue *in vivo*, and show that *in vivo*, at least, these hormones stimulate the enzyme.

EXPERIMENTAL

Animals

Female and male Wistar rats (180–250 g) from A. Tuck and Son (Rayleigh, Essex, U.K.), were fed *ad libitum* on Labsure irradiated CRM diet (Labsure, Poole, Dorset, U.K.). Rats were maintained on a 12 h-light/12 h-dark cycle (light phase 08:00–20:00 h), except for one group which was maintained on a reversed cycle (i.e. darkness 08:00–20:00 h) for 4 weeks before use. All rats were handled regularly before the experiment, and experiments were performed between 12:00 h and 13:00 h unless otherwise indicated.

Studies *in vivo*

All agents were injected intraperitoneally with 26-gauge needles and minimum stress. Agents were obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K. or Boehringer Corp., Lewes, East Sussex, U.K., unless otherwise stated. Rats were anaesthetized with pentobarbital (60 mg/kg body wt.) and remained anaesthetized throughout the experiment. Propranolol (1.5 mg/kg body wt.) was administered along with the pentobarbital, whereas yohimbine (5 mg/kg body wt.) or prazosin (5 mg/kg body wt.) were injected 5 min after the pentobarbital. Prazosin (kindly given by Pfizer Central Research, Sandwich, Kent, U.K.) was dissolved in 40 mM-lactic acid (10 mg/ml) and then diluted to 5 mg/ml with water. Glucose (1 g/kg body wt.), noradrenaline (1.25 mg/kg body wt.) or 0.15 M-NaCl was injected 10 min after the pentobarbital, and 20 min later parametrial white adipose tissue was removed and immediately frozen in liquid N_2 while a blood sample was taken from the heart. Streptozotocin (100 mg/kg body wt.; Upjohn Co., Kalamazoo, MI, U.S.A.) was dissolved in 1 ml of 0.1 M-sodium citrate, pH 4.5, and injected 19 h before the experiment.

Serum was prepared from the blood samples and stored at −20 °C before the insulin assay as described by Vernon *et al.* (1981). Serum glucose was determined with an Analox GM6 glucose analyser.

Studies *in vitro*

Parametrial adipose tissue was removed from virgin rats under pentobarbital anaesthesia, and adipocytes were prepared as described by Rodbell (1964) but using

Abbreviations used: PDH, pyruvate dehydrogenase; PDH_a, pyruvate dehydrogenase in the active state.

Table 1. Effect of noradrenaline *in vivo* and cervical dislocation on the PDH activity of white adipose tissue and the serum insulin concentration of female rats

Rats were either anaesthetized or conscious throughout the whole experimental period. Propranolol was injected along with the anaesthetic; noradrenaline, glucose or NaCl was injected 10 min after the anaesthetic and blood and adipose tissue samples were removed 20 min later. Rats killed by cervical dislocation were conscious at the time; propranolol was given at 30 min and again at 2 min before cervical dislocation. Further details are given in the text. Results are means ± S.E.M., with the numbers of observations in parentheses; *, **, *** indicated that the value is significantly different ($P < 0.05$, < 0.01, < 0.001 respectively) from that for rats given NaCl alone.

Treatment	State of rat	PDH (% in active state)	Serum insulin (ng/ml)
NaCl	Anaesthetized	31.4 ± 3.5 (5)	2.2 ± 0.4
Noradrenaline	Anaesthetized	75.8 ± 9.3 (5)**	3.5 ± 0.6
NaCl + propranolol	Anaesthetized	28.7 ± 0.9 (3)	2.1 ± 0.4
Noradrenaline + propranolol	Anaesthetized	32.9 ± 6.3 (4)	3.3 ± 0.5
Glucose + propranolol	Anaesthetized	70.7 ± 12.3 (3)**	3.9 ± 0.6*
Cervical dislocation	Conscious	81.4 ± 1.2 (4)***	—
Cervical dislocation + propanolol	Conscious	42.0 ± 9.1 (4)	—

1 mg collagenase (type II, Sigma)/ml and suspended in bicarbonate-buffered medium (Krebs & Henseleit, 1932) containing half the original concentration of Ca^{2+}, 5 mM-glucose and 40 mg of dialysed fatty-acid-free albumin (Sigma)/ml, and gassed with O_2/CO_2 (19:1). Samples (300 μl) of the packed cell suspensions were incubated in the presence of agents as described in the Table legends at final concentrations of 30 nM-adrenaline, 50 μunits of insulin/ml, 10 μM-methoxamine, 1 μM-isoprenaline, 10 μM-prazosin, 10 μM-propranolol and 10 μM-yohimbine in a final volume of 1.3 ml. Incubations were carried out at 37 °C in stoppered tubes wrapped in aluminium foil and shaken at 80 oscillations/min for 5 or 30 min. When propranolol, yohimbine or prazosin was used, they were added 2 min before adrenaline. After incubation, cells were separated from the media by layering 250 μl samples on 100 μl of dinonyl phthalate and centrifuging (60 s) in an Eppendorf centrifuge. The cell band was frozen in liquid N_2 before extraction as described for whole adipose tissue for subsequent assay of PDH activity.

Table 2. Effect of noradrenaline on PDH activity of white adipose tissue and serum glucose concentration of streptozotocin-diabetic female rats

Rats were anaesthetized at the start of the experiment. Propranolol was injected along with the anaesthetic, whereas prazosin and yohimbine were injected 5 min after the anaesthetic (administration of these drugs to conscious rats caused obvious stress); noradrenaline or NaCl was injected 10 min after the antagonists, and samples of blood and adipose tissue were removed 20 min later. Further details are given in the text. Results are means ± S.E.M., with the numbers of observations in parentheses; ** value differs ($P < 0.01$) from that for rats receiving NaCl plus propranolol; †† value differs significantly ($P < 0.01$) from that for rats receiving noradrenaline alone.

Treatment	PDH (% in active state)	Serum glucose (mM)
NaCl + propranolol	38.7 ± 2.1 (3)††	28.6 ± 3.0 (3)
Noradrenaline	82.5 ± 9.6 (6)**	27.1 ± 2.4 (6)
Noradrenaline + propranolol	40.3 ± 2.8 (4)††	28.1 ± 4.6 (4)
Noradrenaline + prazosin	34.7 ± 2.4 (3)††	30.6 ± 4.8 (3)
Noradrenaline + yohimbine	54.3 ± 6.3 (3)	34.3 ± 3.3 (3)

PDH assay

Frozen tissue samples were prepared and assayed for PDH activity at 30 °C as described by Stansbie *et al.* (1976*a*), except that the pyruvate and Mg^{2+} concentrations in the assay medium were increased to 3 mM and 2 mM respectively, and 6 mM-pyruvate was added during the activation step before measurement of total PDH activity. The *p*-(*p*-aminophenylazo)benzenesulphonic acid used was kindly given by Dr. R. M. Denton, Department of Biochemistry, University of Bristol.

Results are expressed as means ± S.E.M. and were analysed by Student's *t* test.

RESULTS AND DISCUSSION

Studies *in vivo*

No significant changes in total PDH were observed in response to any of the treatments; the mean total activity for the normal female rats was 144.5 ± 8.2 nmol/min per g wet wt. of tissue (mean ± S.E.M. of 28 observations).

Intraperitoneal injection of noradrenaline increased the proportion of PDH in the active state ($P < 0.01$; Table 1). It took 15 min for the increase in PDH_a to reach a maximum, after which it remained so elevated for at least a further 10 min. This effect was prevented by the prior administration of the β-antagonist propranolol (Table 1). Noradrenaline also appeared to increase the serum insulin concentration (Table 1), probably by increasing the serum glucose concentration, which had risen to 14.8 ± 1.1 mM by 20 min after injection of the catecholamine. Although glucose-induced insulin release

Table 3. Diurnal variation in PDH of white adipose tissue of female rats

All animals were on a 12 h-light/12 h-dark cycle; for rats sampled in the light phase, the light phase began at 08:00 h. Rats sampled in the dark phase were on a reversed lighting regime, with the dark phase commencing at 08:00 h. Rats were anaesthetized 30 min before adipose tissue was removed; propranolol, when given, was injected along with the anaesthetic. Different groups of rats were used in Expts. 1 and 2. Further details are given in the text. Results are means ± S.E.M., with the numbers of observations in parentheses; * value differs significantly ($P < 0.5$) from that for rats at 4 h into the light phase.

Expt.	Condition	Time of day	PDH (% in active state)
1	4 h into light phase	12:00 h	30.6±0.8 (6)
	4 h into dark phase	—	40.5±3.1 (4)*
2	2 h into light phase	10:00 h	47.6±3.2 (4)*
	2 h into light phase +propranolol	10:00 h	28.7±0.9 (3)
	4 h into light phase	12:00 h	31.4±3.5 (5)

is known to increase PDH activity of white adipose tissue (Stansbie *et al.*, 1976*b*; Table 1), this did not seem to be the reason for the effects of noradrenaline observed in the present study, for, in contrast with adipose tissue, injection of the catecholamine had no effect on PDH_a activity of skeletal muscle, whereas injection of glucose increased the activity of PDH_a in this latter tissue (Kilgour & Vernon, 1986). To confirm this point, studies were also carried out with streptozotocin-diabetic rats. Noradrenaline injection was as effective in increasing PDH_a in white adipose tissue from such diabetic rats as in normal rats (Table 2), clearly demonstrating that the effect was not being mediated by an increase in serum insulin. Again the effect of noradrenaline was prevented by propranolol (Table 2). In addition, the α-$_1$-antagonist prazosin also prevented the activation of PDH by noradrenaline in diabetic rats, whereas the α_2-antagonist yohimbine partly prevented this effect of noradrenaline (Table 2). The diabetic state was confirmed in these rats by their high serum glucose concentration (Table 2) and by the occurrence of visually obvious fatty liver.

Perirenal injection of noradrenaline leads to its transport to adipose tissue via the blood, whereas *in vivo* it is released within the tissue itself from sympathetic nerve endings. Severe stress such as cervical dislocation causes a massive surge of sympathetic nervous activity (Callingham & Barrand, 1979). Cervical dislocation of conscious rats resulted in a similar increase in PDH_a activity of white adipose tissue to that found after injection of noradrenaline (Table 1). As before, injection of propranolol before cervical dislocation prevented the rise in PDH_a activity (Table 1).

Further circumstantial evidence for a role of the sympathetic nervous system in modulating PDH activity of white adipose tissue was provided from diurnal changes in the enzyme activity (Table 3). PDH_a activity is higher ($P < 0.05$) during the dark phase than the light phase (Expt. 1, Table 3). PDH_a activity was still elevated 2 h into the light phase (10:00 h), but by 12:00 h it had decreased significantly ($P < 0.05$) (Expt. 2, Table 3). Administration of propranolol to rats before sampling at 10.00 h decreased ($P < 0.05$) the PDH_a activity to that found at 12:00 h (Table 3), suggesting that increased sympathetic activity was responsible for the higher activity at 10:00 h. Sympathetic nervous activity is stimulated by feeding (see Landsberg & Young, 1985); rats, being nocturnal creatures, normally feed and are more active at night, and become quiescent during the day (see Le Magnen, 1984). This pattern of behaviour is reflected in the whole-body oxygen consumption, which tends to increase during the dark phase and then decreases during the first few hours of the light phase (Rothwell & Stock, 1982; Maxwell *et al.*, 1985), at the same time as PDH_a activity decreases.

Noradenaline activated PDH of epididymal (white) adipose tissue of male rats, and this was again blocked by propranolol; results were essentially the same as for female rats (Table 1), and so are not shown. Injection of adrenaline into female rats raised PDH_a activity of parametrial adipose tissue to the same extent as noradrenaline; this effect of adrenaline was also antagonized by either prazosin or propranolol (results not shown).

These various studies show that, *in vivo*, catecholamines activate PDH of white adipose tissue and suggest that changes in sympathetic nervous activity, acting via catecholamines, modulate PDH activity of white adipose tissue.

Studies *in vitro*

The above studies also showed that activation of both α_1- and β-receptors is involved in the stimulation of PDH by noradrenaline. White adipocytes of the rat possess α_1- and β-adrenergic receptors, but not α_2-receptors (see Lafontan & Berlan, 1985), hence the modest inhibitory effects of yohimbine must be indirect. Cheng & Larner (1985), using isolated adipocytes, concluded that catecholamines activate PDH via an α_1-linked mechanism but inhibit via a β-linked mechanism. As our experiments *in vivo* clearly showed that β-blockade with propranolol prevented rather than enhanced the effect of noradrenaline and adrenaline on PDH, we have investigated the effects of catecholamines *in vitro*; adrenaline rather than noradrenaline was used in these studies to facilitate direct comparison with the findings of Cheng & Larner (1985) and also of Smith & Saggerson (1978).

Smith & Saggerson (1978) found that, in the presence of insulin, adrenaline at concentrations below about 100 nM enhanced the PDH_a activity of white adipocytes; at higher concentrations of adrenaline there was a fall in PDH_a activity, with a concomitant rise in the rate of lipolysis. Smith & Saggerson (1978) used a high concentration of insulin (20 munits/ml) in the above experiment. In our system, we found that 200 μunits of insulin/ml (in the absence of adrenaline) had a maximum effect on PDH, increasing the proportion in the active state from 30% to 75%; half-maximum stimulation was achieved with 100 μunits of insulin/ml. In subsequent experiments we used a sub-optimal concentration of insulin of 50 munits/ml; this was sufficient to raise the proportion of PDH in the active state from 30 to 40%. In the presence of 50 μunits of insulin/ml, adrenaline increased the proportion of PDH_a; maximum effect (80% activation) was achieved with 30 nM-adrenaline. At higher concentrations of

Table 4. Effects of propranolol and prazosin on the activation of PDH *in vitro* by adrenaline in white adipocytes from female rats

Adipocytes were incubated for 5 or 30 min in the presence of 5 mM-glucose, 50 μunits of insulin/ml and 30 nM-adrenaline as well as other components as given in the text, in the absence or presence or prazosin (10 μM) or propranolol (10 μM), after which the PDH activity was measured. Adrenaline increased the proportion of PDH_a from 33.7±1.3 to 55.8±2.6% of total PDH activity after 5 min of incubation and from 40.7±2.2 to 75.3±3.5% after 30 min of incubation. Results are expressed as the percentage inhibition of the increase in PDH activity induced by adrenaline, and are means±S.E.M. of three observations.

		PDH (% inhibition of increase in activity induced by adrenaline)	
Antagonist	Incubation . . .	5 min	30 min
Prazosin		58.3±4.8	37.2±0.8
Propranolol		37.2±3.0	75.6±6.4
Prazosin+propranolol		105.2±3.6	123.4±9.2

Table 5. Activation of PDH by the α_1-agonist methoxamine and the β-agonist isoprenaline *in vitro* in white adipocytes from female rats

Adipocytes were incubated for 5 or 30 min in the presence of 5 mM-glucose, 50 μunits of insulin/ml plus methoxamine (10 μM) or isoprenaline (1 μM) along with other ingredients given in the text, after which PDH was assayed. Results are expressed as percentage of PDH in the active state found in the presence of the agonist minus the percentage in its absence. The proportion of PDH in the active state in the absence of agonist was 37.9±8.4 and 42.3±8.4% of total PDH after 5 and 30 min of incubation respectively. Results are means±S.E.M. of three observations in each case.

		Increase in percentage of PDH_a	
Agonist	Incubation . . .	5 min	30 min
Methoxamine		19.8±7.7	10.4±1.3
Isoprenaline		8.3±3.5	26.8±5.8
Methoxamine +isoprenaline		27.8±9.6	36.2±4.1

adrenaline, the proportion of PDH in the active state decreased along with a rise in glycerol release (results not shown). These findings, with a physiological concentration of insulin, are thus in good agreement with those of Smith & Saggerson (1978).

The role of α_1- and β-receptors in the activities of PDH was explored firstly by incubating adipocytes with 30 nM-adenaline and 50 μunits of insulin/ml plus α_1- and β-antagonists. In the preceding experiments, cells were incubated for 30 min (cf. Smith & Saggerson, 1978), as this led to maximal activation. Cheng & Larner (1985) found that the α_1-mediated effects were transient and were lost by 30 min, so we examined the effects of antagonists over 5 min and 30 min incubations; a 5 min incubation increased PDH_a to about 60% of that found after 30 min. Preparatory experiments showed that maximum effects of prazosin and propranolol were achieved at concentrations of 10 μM for both, in agreement with Cheng & Larner (1985). Both prazosin and propranolol inhibited the activation of PDH by adrenaline, and the combination of the two was required to inhibit the activation completely (Table 4). Curiously, the contributions of the α_2- and β-antagonists to the inhibition reversed over the period of incubation with α_1-inhibition predominating at 5 min (in agreement with the findings of Cheng & Larner, 1985), whereas β-inhibition was the major cause at 30 min (Table 4). The α_2-antagonist yohimbine (10 μM) had no effect on PDH activation at either 5 or 30 min (results not shown).

The above findings were confirmed in further experiments using more specific agonists (methoxamine as an α_1-agonist and isoprenaline as a β-agonist). Maximum activation was again achieved with the combination of both agonists (Table 5). Again α_1-activation decreased after 5 min, whereas β-activation increased (Table 5). The concentrations of agonists used were the same as those used by Cheng & Larner (1985): 1 μM-isoprenaline may have stimulated lipolysis, but 1 μM-adrenaline was found to stimulate lipolysis and also activate PDH, albeit sub-optimally.

These studies *in vitro* are thus in agreement with our findings *in vivo* in that catecholamines activated PDH and that this involves both α_1- and β-receptors. The findings differ however in that either α_1- or β-blockade is sufficient to prevent completely the effect of catecholamines on PDH *in vivo*, but both are required *in vitro*.

As noted above, the effects of adrenaline are in agreement with those of Smith & Saggerson (1978), and the finding that activation of PDH is mediated by α_1-receptors agrees with the conclusions of Cheng & Larner (1985). In contrast, our results both *in vitro* and *in vivo* differ from those of Cheng & Larner (1985), as they found that propranolol enhanced the activation caused by α_1-agonists: the reason for this difference is not certain. However, Cheng & Larner (1985) did not normally include insulin in their incubations and used a lower (2 mM) glucose concentration; also the proportion of PDH_a was only 5–10% in their studies, compared with about 30% before stimulation in experiments both *in vitro* and *in vivo* in the present study; Cheng & Larner (1985) attribute the low proportion of PDH_a to the use of 2 mM-glucose in their studies. A lower glucose concentration and the absence of insulin could have led to increased fatty acid accumulation in their adipocytes. Fatty acids are potent inhibitors of PDH (Smith & Saggerson, 1978); hence the increase in PDH_a in the presence of propranolol found by Cheng & Larner (1985) could have arisen from the agent inhibiting lipolysis thereby lowering the intracellular fatty acid concentration, with the concomitant relief of PDH from fatty acid-induced inhibition.

General discussion

Activation of white-adipose-tissue PDH by catecholamines probably relates to the sympathetic nervous system having a more subtle role in the control of body function in addition to its well-defined role in stress. Feeding, for example, results in increased sympathetic

nervous activity, which is even modulated by the nature of the diet (see Landsberg & Young, 1985). Eating promotes triacylglycerol turnover in white adipose tissue *in vivo* (Brooks *et al.*, 1983), almost certainly owing to increased sympathetic nervous activity (the increase was blocked by propranolol). This creates a demand for glycerol 3-phosphate and hence increased glucose metabolism through the initial stages of glycolysis; the rise in phosphofructokinase activity in response to catecholamines (Sale & Denton, 1985) is in accordance with this. Increased triacylglycerol turnover also requires ATP; this could be derived from fatty acid oxidation, but the present study suggests that the increased sympathetic nervous activity (probably in concert with serum insulin, which is also increased on eating) will activate PDH and so increase glucose oxidation (cf. Table 3). Although increased triacylglycerol turnover might be expected to raise the fatty acid concentration in the cell *in vivo*, this is unlikely to be sufficient to affect PDH, for the concentration of catecholamines injected in the present study would markedly raise the plasma fatty acid concentration (Brodie *et al.*, 1965); dissipation of fatty acid may be more difficult for adipocytes *in vitro* than *in vivo*.

Increased triacylglycerol turnover coupled with increased PDH activity and glucose oxidation constitutes an 'ATPase' with the concomitant production of heat. A similar mechanism appears to exist in brown adipose tissue, in which catecholamines also activate PDH *in vivo* (Gibbins *et al.*, 1985). Brown adipose tissue has additional mechanisms for producing heat and is thought to be primarily responsible for the increased heat production found on eating in rats (see Stock & Rothwell, 1986); the present study suggests that white adipose tissue, as a result of increased sympathetic activity, will also make some contribution to increased heat production on eating. In addition, through increased glucose oxidation, the tissue will help to diminish the hyperglycaemia which normally accompanies a meal.

We thank J. McDill for care of the rats. E.K. was in receipt of an A.F.R.C. scholarship.

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Received 24 June 1986/8 August 1986; accepted 19 September 1986