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Regulation of Pyruvate Dehydrogenase Activity in Rat Epididymal Fat-Pads and Isolated Adipocytes by Adrenaline

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1. Dose-dependent effects of adrenaline on PDH_a activity were investigated with both incubated rat epididymal fat-pads and isolated adipocytes. 2. Adrenaline (10nM–5μM) decreased PDH_a activity in fat-pads incubated with 5mM-[U-^{14}C]glucose + insulin (20 munits/ml). Changes in [U-^{14}C]glucose incorporation into fatty acids in these tissues correlated only loosely with changes in PDH_a activity. There was a good inverse relationship between adrenaline-induced changes in PDH_a activity and increases in lipolysis (glycerol release). 3. Adrenaline (10nM–0.5μM) decreased PDH_a activity in fat-pads incubated with 5mM-[U-^{14}C]pyruvate + insulin (20 munits/ml), whereas 1μM- and 5μM-adrenaline slightly increased PDH_a activity. All concentrations of adrenaline tested decreased [U-^{14}C]pyruvate incorporation into fatty acids. Between 10nM- and 0.5μM-adrenaline percentage decreases in PDH_a activity paralleled decreases in fatty acid synthesis. 4. Effects of adrenaline on PDH_a activity and fatty acid synthesis in fat-pads incubated with 5mM-[U-^{14}C]pyruvate + insulin (20 munits/ml) could not be mimicked by addition of albumin-bound palmitate. 5. The response of PDH_a activity to adrenaline (0.1nM–1μM) in isolated adipocytes differed with the carbohydrate substrate used in the incubations. With 5mM-glucose + insulin (20 munits/ml), PDH_a activity was significantly increased by 10nM-adrenaline, but not by 1μM-adrenaline, the response to adrenaline being biphasic. There was some correlation between PDH_a activity and accumulation of non-esterified fatty acids. With 5mM-glucose alone adrenaline (0.1nM–1μM) had no effect on PDH_a activity even though lipolysis was increased by adrenaline (0.1μM–1μM). With 5mM-fructose in the presence and absence of insulin, lipolytic doses of adrenaline decreased PDH_a activity. No tested concentrations of adrenaline increased PDH_a with this substrate. 6. In the presence of 5mM-fructose, palmitate was significantly more effective than adrenaline with respect to the maximum decrease in PDH_a activity that could be elicited. 7. The relationship of changes in PDH_a activity to changes in lipogenesis and the likelihood of adrenaline-induced changes in PDH_a activity being secondary to changes in non-esterified fatty acid metabolism are discussed.

It is well established that the interconversion of the active (PDH_a) and inactive (PDH_b) forms of pyruvate dehydrogenase in rat epididymal adipose tissue can be influenced *in vitro* by hormones. Although the mechanism is still unexplained, there is complete agreement that, whatever the incubation conditions, insulin treatment of the tissue results in an increase, rather than a decrease, in the active form of the enzyme (Jungas, 1970; Coore *et al.*, 1971; Weiss *et al.*, 1971, 1974; Martin *et al.*, 1972; Sica & Cuatrecasas, 1973; Taylor & Jungas, 1974; Severson *et al.*, 1976; Sooranna & Saggerson, 1976*b*). On the other hand, there is complete divergence between the findings of various studies on the actions of lipolytic agents (adrenaline, noradrenaline and corticotropin) on covalent modification of PDH in adipose tissue. For example, Jungas (1971), Sica & Cuatrecasas (1973), Weiss *et al.* (1974) and Taylor *et al.* (1973) have all reported PDH_a activity to be increased after exposure of fat-pads to lipolytic agents in the absence of insulin, whereas Coore *et al.* (1971), who observed no effect of adrenaline alone (except at very short incubation times), found that various lipolytic agents could oppose the increase in PDH_a activity caused by insulin. Weiss *et al.* (1974) similarly found that noradrenaline could decrease PDH_a activity only in insulin-stimulated adipose tissues. With isolated fat-cells, however, considerable decreases in PDH_a activity are found with adrenaline alone (Coore *et al.*, 1971; Sooranna & Saggerson, 1976*a*). Interpretation of these apparently contradictory findings is complicated by the fact that some incuba-

Abbreviations used: PDH, pyruvate dehydrogenase; PDH_a and PDH_b, active and inactive forms respectively of pyruvate dehydrogenase.

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tions contained no added substrates or only pyruvate, whereas others contained fructose or glucose, which permit some re-esterification of lipolysis-derived fatty acids. Further, albumin was absent from incubation media in some studies, but present in others. It is therefore very difficult to correlate all the findings of these studies and deduce what changes in PDH_a activity are likely to be secondary to fatty acid accumulation and which could be due to some other action of the lipolytic hormones in question. PDH_a activity in liver, heart and kidney is decreased with increasing extracellular fatty acid concentrations (Wieland *et al.*, 1971, 1972; Guder *et al.*, 1974). Also it has been shown that fatty acids similarly decrease PDH_a activity in isolated adipocytes (Sooranna & Saggerson, 1976*b*).

Very high concentrations of lipolytic agents were generally used in previous studies (see references above), and, with one exception (Sooranna & Saggerson, 1976*a*), no dose responses for actions of lipolytic agents on adipose-tissue PDH_a activity appear to have been published. It was therefore the purpose of this study to examine the dose-dependence of adrenaline actions on PDH_a activity under various conditions. The changes observed in PDH_a activity have been compared with the accompanying lipolytic response to attempt to establish how closely these are related, and whether the former might be secondary to the latter. In some instances dose-dependent changes in PDH_a activity have also been compared with simultaneous measurements of fatty acid synthesis to establish how closely PDH covalent modification is involved in the regulation of lipogenesis.

Materials and Methods

Chemicals

ADP, NAD^+, NADH and collagenase (type I from *Clostridium histolyticum*) were obtained from International Enzymes Ltd. (Windsor, Berks., U.K.), and sodium pyruvate, 2-oxoglutarate, CoA and all enzymes not otherwise indicated were from Boehringer Corporation (London) Ltd. (Lewes, Sussex, U.K.). Bovine plasma albumin powder (fraction V), DNA (type V, sodium salt, highly polymerised; from calf thymus) and L-adrenaline were from Sigma (London) Chemical Co. (Kingston upon Thames, Surrey, U.K.). Thiamin pyrophosphate was from Calbiochem Ltd. (Hereford, U.K.), sodium palmitate from Nu Chek Prep (Elysian, MI, U.S.A.) and *p*-aminoazobenzenesulphonic acid from Fluka (Buchs, Switzerland). [U-^{14}C]Glucose and [U-^{14}C]pyruvate were purchased from The Radiochemical Centre (Amersham, Bucks., U.K.) and 2,5-bis-(5-t-butylbenzoxazol-2-yl)thiophen was from CIBA (A.R.L.) Ltd. (Duxford, Cambs., U.K.). Bovine insulin (six times recrystallized) was from Boots Pure Drug Co. Ltd. (Nottingham, U.K.) and anti-insulin serum from the Wellcome Research Laboratories (Beckenham, Kent, U.K.).

Preparative procedures

Arylamine acetyltransferase (EC 2.3.1.5) was prepared from pigeon liver as described by Coore & Field (1974). Fraction V albumin was subjected to a defatting procedure (Chen, 1967), with the minor modifications described by Saggerson (1972). Sodium palmitate was associated with fatty acid-poor albumin as described by Evans & Mueller (1963) and standardized by the colorimetric method of Itaya & Ui (1965).

Animals

These were male Sprague–Dawley rats bred in the animal colony at University College London, and maintained on GR3EK diet (E. Dixon and Sons, Ware, Herts., U.K.) until the time of experimentation, when they weighed 160–180g.

Incubation of and preparation of extracts from fat-pads

Rats were killed by cervical dislocation, the epididymal adipose tissue was excised, trimmed free of major blood vessels, and the fat-pads were collected in Krebs–Ringer bicarbonate buffer at 37°C (Krebs & Henseleit, 1932). The tissues from four rats were distributed in a paired fashion between two 50ml incubation flasks ensuring that each flask received two left and two right fat-pads. Flasks containing adrenaline were wrapped in metal foil to exclude light. A preliminary incubation was performed for 30min in 10ml of Krebs–Ringer bicarbonate buffer containing 1.27mM-$CaCl_2$, fatty acid-poor albumin (12mg/ml) and either unlabelled glucose (5mM) or unlabelled sodium pyruvate (5mM) as appropriate. Other additions are indicated in individual Tables. The flask were sealed and shaken (approx. 70 oscillations per min) under O_2/CO_2 (19:1). After 30min, the tissues were transferred to flasks containing fresh incubation media with identical additions, except that [U-^{14}C]glucose or [U-^{14}C]pyruvate (0.1μCi/ml) was added as appropriate. After a further 30min of incubation, the tissues were removed from the flask, lightly blotted and rapidly frozen in liquid N_2. The frozen tissues were wrapped in aluminium foil and kept under liquid N_2 for up to 4h before further use. Individual lots of four frozen fat-pads were then finely powdered in a mortar under liquid N_2. When ^{14}C-labelled lipid synthesis was measured a small weighed portion of this powder (approx. 100mg) was transferred to 20ml of propan-2-ol + hexane + 0.5M-H_2SO_4 (40:

10:1, by vol.) and the radioactive lipids were extracted into hexane (Dole, 1956). The remainder of the frozen powder was homogenized in 5ml of 20mM-potassium phosphate (adjusted to pH7.0 with HCl) containing 2mM-EDTA (Coore *et al.*, 1971) in an Ultra-Turrax tissue disintegrator (Jahnke und Kunkel, Staufen, German Federal Republic). This was performed in tubes pre-cooled in an ice/salt mixture (−6 to −10°C). The extracts were centrifuged at $1000g_{av.}$ for 10min at 4°C and samples of the resulting fat-free infranatant taken for assay of enzymes.

Preparation, incubation and extraction of fat-cells

The pooled epididymal fat-pads of four rats were disaggregated with collagenase as described by Rodbell (1964). The cells were washed with Krebs–Ringer bicarbonate buffer containing 1.27mM-Ca^{2+} and fatty acid-poor albumin (10mg/ml) and made up to 12ml. Portions (2ml) of this stock suspension were used for determination of DNA, and 1ml portions were added to 25ml silicon-treated flasks and incubated at 37°C with shaking under O_2/CO_2 (19:1) in a final volume of 4ml of Krebs–Ringer bicarbonate buffer containing 1.27mM-Ca^{2+}, fatty acid-poor albumin (32.5mg/ml) and other additions indicated in the Figure legends. Flasks containing adrenaline were wrapped in metal foil to exclude light. After 30min the cells were separated from the incubation media by centrifugation (Sooranna & Saggerson, 1976*b*) and frozen in liquid N_2. The cells were stored for up to 4h under liquid N_2 before being extracted with 1ml of 20mM-potassium phosphate (adjusted to pH7.0 with HCl) containing 2mM-EDTA as described by Sooranna & Saggerson (1976*a*). The fat-cell homogenates were centrifuged for 30s in an Eppendorf 3200 centrifuge and the resulting infranatant was used for assay of enzymes.

Analytical methods

Pyruvate dehydrogenase (EC 1.2.4.1) was assayed spectrophotometrically at 25°C by coupling to arylamine acetyltransferase (Coore *et al.*, 1971). Glutamate dehydrogenase (EC 1.4.1.2) was assayed as described by Martin & Denton (1970).

Extracts of fat-pads in hexane were analysed for ^{14}C incorporation into glyceride fatty acids and glyceride glycerol as described by Saggerson & Greenbaum (1970) and Saggerson & Tomassi (1971).

Incubation me[illegible] recovered after incubation of fat-pads or fat-cells were stored frozen at −20°C. Subsequently, portions were deproteinized (Saggerson, 1972) and assayed for glycerol by the method of Garland & Randle (1962) unless high concentrations of pyruvate were present, in which case the method of Wieland (1957) was used. Further 2ml portions of incubation media were taken and non-esterified fatty acids extracted into hexane (Saggerson & Tomassi, 1971). Duplicate portions of these hexane extracts were then evaporated to dryness at 60–70°C, 6ml of chloroform was added and fatty acids were determined as described by Itaya & Ui (1965) with palmitic acid as a standard.

Fat-cell DNA was measured as described by Saggerson (1972).

Statistical methods

Analysis of data was performed on a paired basis and statistical significance determined by Student's *t* test.

Results and Discussion

Experiments with incubated epididymal fat-pads

To attempt to compare changes in PDH_a activity with changes in other metabolic parameters, fat-pads were preincubated for 30min in the absence of radioactive label to establish a steady state, which was then continued for a further 30min in the presence of ^{14}C-labelled substrates (see the Materials and Methods section). PDH_a measurements, which were made in the same tissues as those used for other measurements, refer to enzyme activity at the end of this second 30min period.

Table 1, Expt. 1, shows that, in the presence of 5mM-glucose, insulin (20 munits/ml) increased PDH_a activity approx. 4-fold whereas fatty acid synthesis from [U-^{14}C]glucose was increased more than 9-fold. Expts. 2–7 (Table 1) provided the data to construct dose–response curves over the adrenaline concentration range 10nM–5μM. Insulin (20 munits/ml) was present throughout to ensure high PDH_a activities in control incubations. Significant increases in lipolysis (as measured by glycerol release) were apparent at, and above, 0.1μM-adrenaline. This was accompanied by a significant increase in glyceride glycerol formation, indicating more fatty acid re-esterification. At 0.1μM-adrenaline there was a small, but significant, increase in fatty acid synthesis; this is also seen with low concentrations of adrenaline when adipocytes are incubated with [U-^{14}C]glucose + insulin (Saggerson, 1972). At 0.5μM and higher concentrations of adrenaline, glyceride glycerol formation approached its maximum rate and this was accompanied by significant non-esterified fatty acid accumulation in incubation media, decreased fatty acid synthesis and decreased PDH_a activity. The greatest accumulation of non-esterified fatty acids (at 5μM-adrenaline) was 381 ± 14μM, which was equivalent to only 2.1mol of fatty acid/mol of albumin in the incubation media. Glutamate dehydrogenase activity was measured in all tissue

Table 1. *Effect of insulin and adrenaline on PDH_a activity, lipogenesis and lipolysis in fat-pads incubated with glucose*

Fat-pads were preincubated for 30 min as described in the Materials and Methods section and then incubated for a further 30 min in Krebs–Ringer bicarbonate buffer containing fatty acid-poor albumin (12 mg/ml) and 5 mM-[U-^{14}C]glucose. Where indicated insulin was added at 20 munits/ml (1 unit is 41.7 μg). The results are means ± S.E.M.; **, ***, **** indicate $P < 0.02$, 0.01, 0.001 respectively for comparison against the paired controls, which in every case were the incubations with glucose and insulin alone.

Experiment no.	Additions to incubation	Mean wet wt. of tissue incubated (g)	PDH_a activity (μmol/min per g wet wt.)	Glutamate dehydrogenase activity (μmol/min per g wet wt.)	[U-^{14}C]Glucose incorporation (μg-atoms/30 min per g wet wt.)		Non-esterified fatty acid release (μmol/30 min per g wet wt.)	Glycerol release (μmol/30 min per g wet wt.)	No. of observations
					Fatty acids	Glyceride glycerol			
1.	Insulin	1.40 ± 0.09	0.227 ± 0.021	0.98 ± 0.06	12.18 ± 0.54	1.43 ± 0.11	0.26 ± 0.02	0.28 ± 0.03	5
	None	1.41 ± 0.09	0.055 ± 0.010****	0.96 ± 0.09	1.30 ± 0.26****	0.44 ± 0.05****	0.47 ± 0.05***	0.66 ± 0.08***	
2.	Insulin	1.44 ± 0.06	0.259 ± 0.012	0.92 ± 0.08	14.02 ± 1.26	1.74 ± 0.22	0.15 ± 0.02	0.29 ± 0.02	7
	Insulin + adrenaline (0.01 μM)	1.44 ± 0.05	0.255 ± 0.011	0.94 ± 0.07	13.68 ± 1.40	1.78 ± 0.27	0.15 ± 0.02	0.27 ± 0.02	
3.	Insulin	1.25 ± 0.06	0.310 ± 0.019	1.23 ± 0.09	14.95 ± 1.13	1.93 ± 0.13	0.14 ± 0.003	0.25 ± 0.04	4
	Insulin + adrenaline (0.03 μM)	1.29 ± 0.08	0.299 ± 0.005	1.14 ± 0.07	15.37 ± 0.99	2.22 ± 0.24	0.14 ± 0.01	0.31 ± 0.06	
4.	Insulin	1.15 ± 0.06	0.249 ± 0.024	0.99 ± 0.06	12.70 ± 1.12	1.86 ± 0.13	0.29 ± 0.07	0.34 ± 0.04	4
	Insulin + adrenaline (0.1 μM)	1.17 ± 0.04	0.236 ± 0.021	1.00 ± 0.04	13.99 ± 1.11**	4.64 ± 0.25***	0.42 ± 0.04	1.72 ± 0.06****	
5.	Insulin	1.17 ± 0.05	0.251 ± 0.038	0.95 ± 0.05	13.59 ± 0.59	1.94 ± 0.12	0.27 ± 0.08	0.39 ± 0.04	4
	Insulin + adrenaline (0.5 μM)	1.20 ± 0.03	0.184 ± 0.034***	1.01 ± 0.04	9.38 ± 0.61***	9.93 ± 0.49***	1.97 ± 0.07****	5.88 ± 0.20****	
6.	Insulin	1.07 ± 0.01	0.271 ± 0.014	1.13 ± 0.01	15.93 ± 1.29	2.27 ± 0.07	0.19 ± 0.02	0.38 ± 0.04	4
	Insulin + adrenaline (1 μM)	1.05 ± 0.04	0.171 ± 0.007***	1.14 ± 0.04	8.68 ± 0.35***	11.46 ± 0.40****	3.34 ± 0.17****	7.87 ± 0.75***	
7.	Insulin	1.20 ± 0.07	0.258 ± 0.026	1.05 ± 0.10	14.23 ± 1.04	2.30 ± 0.07	0.17 ± 0.02	0.35 ± 0.02	4
	Insulin + adrenaline (5 μM)	1.20 ± 0.07	0.166 ± 0.014***	0.98 ± 0.08	5.59 ± 0.54****	9.92 ± 0.62***	3.18 ± 0.06****	7.34 ± 0.51****	

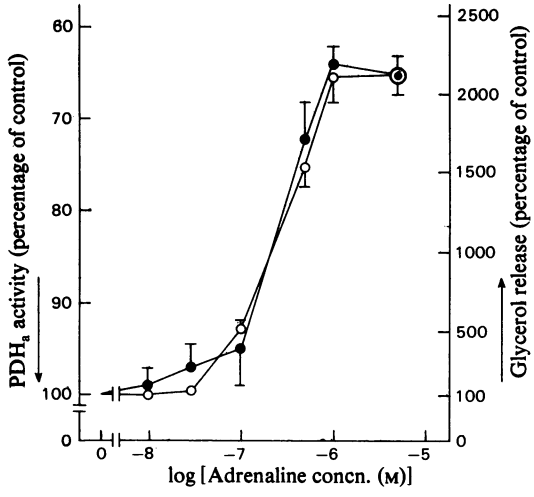

Fig. 1. *Percentage changes in PDH_a activity and glycerol release in fat-pads incubated with glucose and insulin and treated with adrenaline*

The data are derived from Table 1 and the scales normalized by making the 5 μM-adrenaline values equivalent. The bars represent S.E.M. ○, Glycerol release; ●, PDH_a activity.

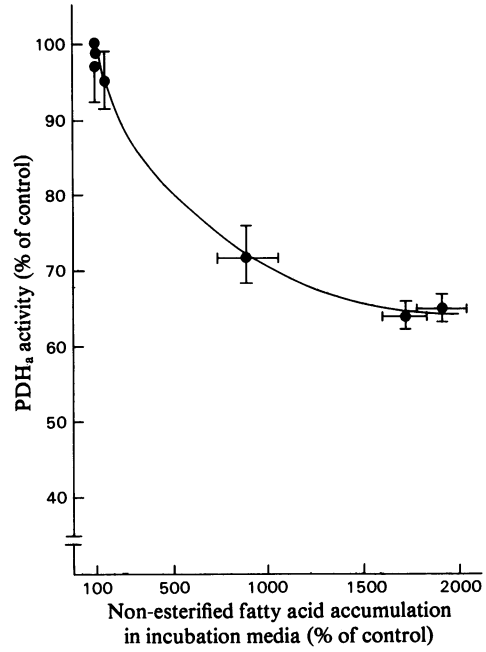

Fig. 2. *Relationship between percentage changes in non-esterified fatty acid accumulation in incubation media and in PDH_a activity in fat-pads incubated with glucose and insulin and treated with adrenaline*

The data are derived from Table 1. The bars represent S.E.M.

extracts in these experiments. It may be seen (Table 1) that its extraction by Ultra-Turrax homogenization was very constant and that the hormonal treatments did not lead to any alteration in enzyme activity. Since extraction of this mitochondrial matrix marker enzyme was reproducible, it was thought reasonable to express fat-pad PDH_a activities in subsequent experiments per g wet weight of tissue, particularly since we wished to compare this parameter with flux rates expressed on the same basis (see below). Expts. 2–7 (Table 1) confirm the findings of Coore *et al.* (1971) and Weiss *et al.* (1974) that lipolytic agents can decrease PDH_a in fat-pads incubated with insulin and a sugar substrate. These results show, however, that the maximum effect of the lipolytic agent is only a partial reversal of the insulin-promoted increase in activity (cf. Expts. 7 and 1 in Table 1). This is in accord with the findings of Weiss *et al.* (1974), who also used glucose as substrate, but differs from those of Coore *et al.* (1971), who observed almost complete abolition of the insulin-promoted increase in PDH_a activity after treatment of fat-pads with lipolytic agents in the presence of fructose.

Figs. 1–3 show data derived from Expts. 2–7 of Table 1 by expression of values as percentages of their paired controls, which contained insulin but no adrenaline. Fig. 1 shows that the normalized dose-dependencies of adrenaline actions to decrease PDH_a activity and to increase glycerol release are very similar. This may be approached in another way: an inverse relationship between PDH_a activity and lipolysis (glycerol release) is also seen if all the 27 individual values for PDH_a activity and glycerol release in the presence of adrenaline are expressed as percentages of their paired controls and a linear-regression analysis is performed using the two variables. This gave a correlation coefficient (r) of -0.922 ($P < 0.01$). Although other mechanisms have been advanced to explain the effects of adrenaline on PDH_a activity (Severson *et al.*, 1976), we suggest that, in view of the known relationship between PDH_a activity and fatty acid concentration (see the introduction for references), this close relationship between the lipolytic action of adrenaline and the decrease in PDH_a implies that the latter may be secondary to the former.

Fig. 2 shows the relationship between percentage changes in PDH_a activity and non-esterified fatty acid accumulation in incubation media as a result of adrenaline stimulation.

Fig. 3 shows that changes in PDH_a activity caused by adrenaline did not correlate particularly closely with changes in fatty acid synthesis. This was particularly apparent at higher adrenaline concentrations. However, previous studies have shown that

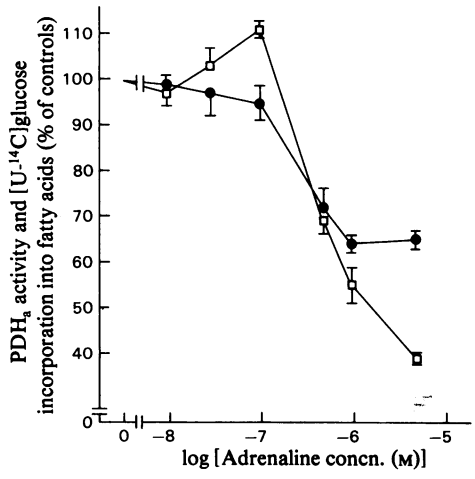

Fig. 3. *Comparison of percentage changes in [U-^{14}C]-glucose incorporation into fatty acids and in PDH$_a$ activity in fat-pads incubated with glucose and insulin and treated with adrenaline*

The data are derived from Table 1. The bars represent S.E.M. ●, PDH$_a$ activity; □, fatty acid synthesis.

fat-pads incubated with [^{14}C]glucose, insulin and adrenaline have increased flux of glucose carbon into tricarboxylic acid-cycle CO_2 compared with controls incubated with insulin alone (Flatt & Ball, 1964; Saggerson & Greenbaum, 1970). Thus the smaller percentage decrease in PDH$_a$ activity compared with the decrease in fatty acid synthesis is to compensate for the increase in tricarboxylic acid-cycle rate. Also, Expt. 1 in Table 1 shows that, although they are similar in direction, the percentage increases in PDH$_a$ activity and fatty acid synthesis resulting from insulin stimulation do not correlate, the latter being approximately twice the former. Previous calculations, however, have shown that, in the absence of insulin, tricarboxylic acid-cycle CO_2 formation accounts for an appreciable proportion of glucose-carbon flux to acetyl-CoA via PDH (10% in Flatt & Ball, 1964; 28% in Katz *et al.*, 1966; and 23% in Saggerson & Greenbaum, 1970). Insulin greatly stimulates fatty acid synthesis from glucose, but has little effect on appearance of glucose carbon in tricarboxylic acid-cycle CO_2 (Flatt & Ball, 1964; Katz *et al.*, 1966; Saggerson & Greenbaum, 1970). Therefore in the presence of insulin alone tricarboxylic acid-cycle CO_2 formation accounts for a considerably smaller proportion of glucose-carbon flux to acetyl-CoA via PDH (3.3% in Flatt & Ball, 1964; 7.7% in Katz *et al.*, 1966; 2.2% in Saggerson & Greenbaum, 1970). *In vivo* also, Stansbie *et al.* (1976) have observed parallel changes in rat epididymal fat-pad PDH$_a$ activity and ^3H_2O incorporation into fatty acids after injection of glucose or anti-insulin serum. Again it may be seen from these data that percentage changes in PDH$_a$ activity are less than those in fatty acid synthesis (particularly when PDH$_a$ measurements after 30min of treatment are compared with fatty acid synthesis over 1h). It is suggested that, because PDH plays a 'bioenergetic' role as well as supplying acetyl precursors for lipogenesis, changes in PDH$_a$ activity can only act as a 'coarse' control of lipogenesis and that there is need for further fine control(s) in the pathway between pyruvate and fatty acids.

Expts. 1–5 of Table 2 provide data to construct dose–response curves over the adrenaline concentration range 30nM–5μM when 5mM-pyruvate and insulin (20 munits/ml) were present.

Pyruvate was deliberately chosen for these experiments because, although it may act readily as a fatty acid precursor (see Saggerson, 1974), it is a very ineffective precursor for glyceride glycerol formation (Saggerson & Tomassi, 1971; and Table 2) and hence re-esterification of released fatty acids is very low in the presence of this substrate. It was established in preliminary experiments that insulin (20 munits/ml) approximately doubled PDH$_a$ activity in the presence of 5mM-pyruvate and increased [^{14}C]pyruvate incorporation into fatty acids by 44%. Adrenaline elicited non-esterified fatty acid accumulation in amounts that were not dissimilar to those in the previous experiments with glucose (Table 1). However, glycerol release was considerably lower in the presence of pyruvate. In the absence of active re-esterification, it may be reasonable to expect intracellular non-esterified fatty acid concentrations to be higher with pyruvate than with glucose. This might account for the lower glycerol release with pyruvate since long-chain fatty acids appear to have a feedback inhibitory effect on the hormone-sensitive triacylglycerol lipase (Fain & Shepherd, 1976).

Table 2 shows that with increasing adrenaline concentration fatty acid synthesis from [U-^{14}C]-pyruvate decreased and lipolysis (as measured by glycerol release or non-esterified fatty acid accumulation) increased. [^{14}C]Pyruvate incorporation into fatty acids has been shown to be similarly decreased by adrenaline or extracellular fatty acids in rat adipocytes (Saggerson, 1972; Saggerson & Tomassi, 1971). Fig. 4 shows, however, that percentage changes in PDH$_a$ activity only paralleled changes in fatty acid synthesis between 30nM- and 0.5μM-adrenaline. At 1μM- and 5μM-adrenaline PDH$_a$ activity was appreciably increased above the values seen in the paired controls and these changes were clearly dissociated from changes in fatty acid synthesis, again indicating that PDH covalent modification and control of lipogenesis need not be tightly linked. Increases in PDH$_a$ activity after treatment with high concentrations of adrenaline (2μM in Jungas, 1971;

Table 2. *Effect of adrenaline on PDH_a activity, lipogenesis and lipolysis in fat-pads incubated with pyruvate*

Fat-pads were preincubated for 30min as described in the Materials and Methods section and then incubated for a further 30min in Krebs–Ringer bicarbonate buffer containing fatty acid-poor albumin (12mg/ml), 5mM-sodium [U-^{14}C]pyruvate and insulin (20 munits/ml). The results are means ± S.E.M; *, **, ***, **** indicate $P < 0.05$, <0.02, <0.01, <0.001 respectively for comparison against paired control incubations with pyruvate and insulin alone.

Experiment no.	Additions to incubation	Mean wet weight of tissue (g)	PDH_a activity (μmol/min per g wet wt.)	[U-^{14}C]Pyruvate incorporation (μg-atoms/30min per g wet wt.)		Non-esterified fatty acid release (μmol/30min per g wet wt.)	Glycerol release (μmol/30min per g wet wt.)	No. of determinations
				Fatty acids	Glyceride glycerol			
1.	Insulin	1.76 ± 0.13	0.073 ± 0.004	2.32 ± 0.07	0.18 ± 0.02	0.33 ± 0.02	0.14 ± 0.01	4
	Insulin + adrenaline (0.03 μM)	1.73 ± 0.10	0.068 ± 0.006	2.29 ± 0.14	0.16 ± 0.01	0.37 ± 0.03	0.20 ± 0.02*	
2.	Insulin	1.41 ± 0.06	0.061 ± 0.003	2.62 ± 0.15	0.31 ± 0.06	0.30 ± 0.04	0.15 ± 0.02	8
	Insulin + adrenaline (0.1 μM)	1.45 ± 0.05	0.047 ± 0.006*	2.03 ± 0.15*	0.24 ± 0.02	0.59 ± 0.10***	0.31 ± 0.05***	
3.	Insulin	1.37 ± 0.07	0.065 ± 0.005	3.20 ± 0.36	0.25 ± 0.03	0.24 ± 0.05	0.13 ± 0.02	6
	Insulin + adrenaline (0.5 μM)	1.43 ± 0.08	0.047 ± 0.006***	1.99 ± 0.13***	0.34 ± 0.04	1.29 ± 0.26**	0.67 ± 0.22*	
4.	Insulin	1.26 ± 0.03	0.077 ± 0.008	3.46 ± 0.46	0.27 ± 0.03	0.25 ± 0.02	0.15 ± 0.01	7
	Insulin + adrenaline (1 μM)	1.29 ± 0.03	0.106 ± 0.019	1.54 ± 0.19***	0.29 ± 0.04	2.53 ± 0.03****	0.86 ± 0.20***	
5.	Insulin	1.24 ± 0.03	0.087 ± 0.008	2.82 ± 0.34	0.20 ± 0.02	0.33 ± 0.04	0.15 ± 0.02	7
	Insulin + adrenaline (5 μM)	1.26 ± 0.03	0.098 ± 0.018	1.08 ± 0.15****	0.22 ± 0.04	3.36 ± 0.03****	1.40 ± 0.37**	

approx. 5.5μM in Sica & Cuatrecasas, 1973) are also seen in the absence of any added substrates. This is a similar experimental situation to that used here when pyruvate was substrate, the tissue having no capacity to re-esterify the fatty acid products of lipolysis in contrast with the case when glucose or fructose is present.

It is noteworthy that decreases, but not increases, in PDH_a activity were seen when rat adipocytes were incubated in the presence of albumin, but without substrates and with various adrenaline concentrations up to 5μM (Sooranna & Saggerson, 1976*a*). It is suggested that the increases in PDH_a observed in some previous studies either resulted from total or near lack of albumin in the incubations (Sica & Cuatrecasas, 1973; Taylor *et al.*, 1973) or may be an artifact of the incubated fat-pad system where the extent of fatty acid accumulation in the extracellular spaces of the tissue cannot be ascertained.

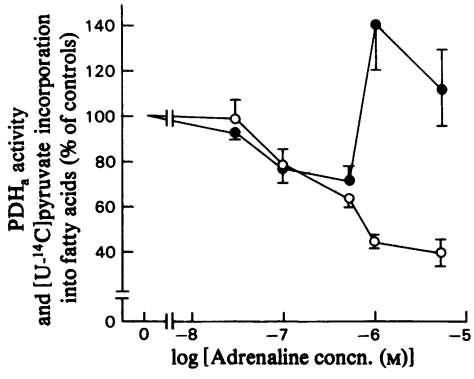

Fig. 4. *Comparison of percentage changes in [U-^{14}C]-pyruvate incorporation into fatty acids and in PDH_a activity in fat-pads incubated with pyruvate and insulin and treated with adrenaline*

The data are derived from Table 2. The bars represent S.E.M. ●, PDH_a activity; ○, fatty acid synthesis.

In isolated adipocytes addition of extracellular fatty acid can mimic the effects of adrenaline on fatty acid synthesis from [U-^{14}C]pyruvate (Saggerson, 1972). In addition, adipocyte PDH_a is decreased by palmitate in the presence of no added substrates or fructose (Sooranna & Saggerson, 1976*b*), pyruvate or glucose (S. R. Sooranna & E. D. Saggerson, unpublished work). The experiments shown in Table 3 were unsuccessful attempts to mimic the adrenaline effects shown in Table 2 by addition of palmitate. In the experiments summarized in Table 2 non-esterified fatty acid accumulation in incubation media ranged from 40 ± 3μM (mean ± S.E.M. of all control data) to 420 ± 39μM (with 5μM-adrenaline). The additions of palmitate shown in Table 3 are therefore similar to this range of fatty acid accumulation but are clearly ineffective in mimicking adrenaline effects either on PDH_a activity or on fatty acid synthesis. It is suggested that this could be attributed to failure of albumin-bound palmitate to penetrate the spaces of the tissue mass and that the incubated fat-pad preparation may be inadequate for this kind of experiment. Further experiments were therefore performed with isolated adipocytes. In this prepara-

Table 3. *Effect of palmitate on PDH_a activity and fatty acid synthesis in fat-pads incubated with pyruvate*

Fat-pads were preincubated for 30min as described in the Materials and Methods section and then incubated for a further 30min in Krebs–Ringer bicarbonate buffer containing fatty acid-poor albumin (12mg/ml), 5mM-sodium [U-^{14}C]pyruvate, insulin (20 munits/ml) and the indicated concentrations of sodium palmitate. The results are means ± S.E.M.; * indicates $P < 0.05$ for comparison against the paired control with pyruvate and insulin alone. Values in parentheses are percentages compared with these paired controls.

Experiment no.	Additions to incubation	Mean wet weight of tissue (g)	PDH_a (μmol/min per g wet wt.)	[U-^{14}C]Pyruvate incorporation into fatty acids (μg-atoms/30min per g wet wt.)	No. of determinations
1.	Insulin	0.92 ± 0.04	0.028 ± 0.004	4.85 ± 0.74	8
	Insulin + palmitate (0.07mM)	0.95 ± 0.04	0.021 ± 0.003 (79 ± 9%)	4.20 ± 0.60* (88 ± 4%)	
2.	Insulin	1.04 ± 0.04	0.027 ± 0.006	5.16 ± 0.42	4
	Insulin + palmitate (0.16mM)	1.04 ± 0.04	0.025 ± 0.005 (100 ± 17%)	4.80 ± 0.29 (94 ± 5%)	
3.	Insulin	0.93 ± 0.02	0.049 ± 0.007	—	3
	Insulin + palmitate (0.37mM)	0.90 ± 0.03	0.039 ± 0.010 (77 ± 10%)	—	
4.	Insulin	0.83 ± 0.03	0.036 ± 0.009	5.86 ± 0.53	5
	Insulin + palmitate (0.65mM)	0.84 ± 0.03	0.028 ± 0.005 (98 ± 18%)	5.11 ± 0.83 (89 ± 14%)	

tion problems of access of extracellular fatty acids are abolished and, unlike the fat-pad preparation, the concentration of fatty acids in the extracellular space (the incubation medium) can be readily ascertained.

Experiments with incubated adipocytes

Dose relationships between adipocyte PDH_a activity or lipolysis and adrenaline concentration are shown in Figs. 5(*a*)–5(*c*). Since lipogenesis was not measured in these experiments incubations were only performed for one 30min period. Also a higher albumin concentration was used in these incubation media than was used in the experiments with incubated fat-pads. To correct for any incompleteness in recovery of cells from incubation media PDH_a activities are expressed as PDH_a/glutamate dehydrogenase activity ratios. When 5mM-glucose and insulin (20 munits/ml) were present low concentrations of adrenaline significantly increased PDH_a activity ($P < 0.05$ at 3nM-, 10nM- and 30nM-adrenaline). The peak of this increase was seen at 10nM and was 41% over the control without adrenaline. There was no change in glycerol release in this adrenaline concentration range, but low concentrations of adrenaline actually decreased non-esterified acid release ($P < 0.01$ at 0.1nM, 1nM, 3nM). The explanation for this phenomenon is unclear, but the results suggest an effect of low concentrations of adrenaline to stimulate fatty acid re-esterification. Adrenaline between 30nM and 1μM caused a dose-dependent decrease in PDH_a activity from the peak value seen at 10nM. Between 0.1μM- and 1μM-adrenaline this decrease in PDH_a activity was accompanied by increased glycerol and non-esterified fatty acid release into the incubation media. No tested concentration of adrenaline decreased PDH_a to below the control value, which is at variance with the observations made in fat-pads incubated under similar conditions (Table 1 and Fig. 3). It is suggested that incubated fat-pads may retain traces of circulatory catecholamines and must also contain nerve endings capable of catecholamine release. Therefore PDH_a activity in fat-pads incubated with glucose and insulin alone may already be sufficiently

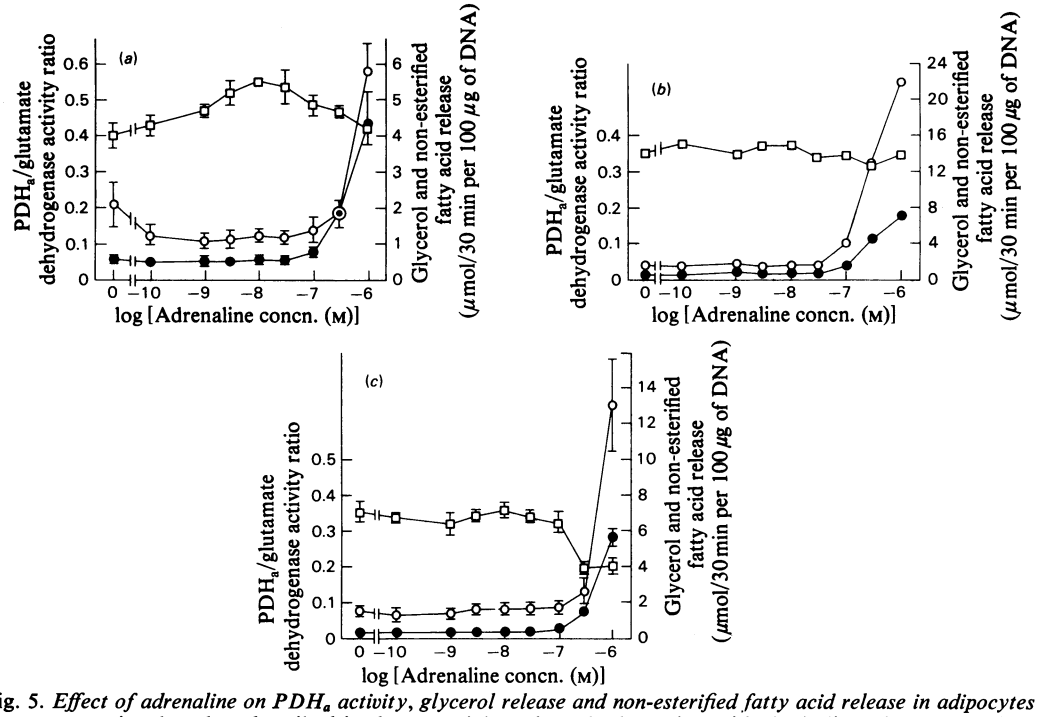

Fig. 5. *Effect of adrenaline on PDH_a activity, glycerol release and non-esterified fatty acid release in adipocytes*
Adipocytes were incubated as described in the Materials and Methods section with the indicated concentrations of adrenaline and other additions shown below. The bars shown in (*a*) and (*c*) represent S.E.M. No error bars are shown in (*b*). □, PDH_a/glutamate dehydrogenase activity ratio; ●, glycerol release; ○, non-esterified fatty acid release. (*a*) With 5mM-glucose + insulin (20 munits/ml), four independent observations (mean fat-cell DNA was 5.0μg/ml of flask contents). (*b*) With 5mM-glucose, three independent observations (mean fat-cell DNA was 7.4μg/ml of flask contents). (*c*) With 5mM-fructose + insulin (20 munits/ml), four independent observations (mean fat-cell DNA was 6.7μg/ml of flask contents).

stimulated by endogenous catecholamine so that only decreases relative to this control are seen on addition of exogenous adrenaline. This effect of low apparently non-lipolytic doses of adrenaline to increase PDH_a activity has not been reported before. Previous reports of increases in PDH_a activity after administration of adrenaline (see the introduction for references) concern experiments with very high lipolytic doses of hormone. Fig. 5(*a*) shows some degree of inverse correlation between extracellular accumulation of fatty acids and PDH_a activity, but this is by no means complete. Unfortunately the relationship between intracellular and extracellular non-esterified fatty acid accumulation is unknown.

Although catecholamine stimulation of rat adipose-tissue lipolysis is generally held to be only through a β-adrenergic receptor, lipolysis is decreased by catecholamines in the presence of a β-blocker, and conversely α-blockade appears to increase the lipolytic response to catecholamines (see review by Himms-Hagen, 1970). Also Perry & Hales (1970) reported an α-adrenergic effect on $^{45}K^+$ efflux from rat adipocytes. At present we have no evidence that would indicate whether the effects reported above are solely β-adrenergic or possibly are attributable to both α and β effects.

Fig. 5(*b*) shows that, when adipocytes were incubated with glucose alone, adrenaline (0.1 nM–1 μM) had no appreciable effect on PDH_a activity even in the range 0.1–1 μM where increased lipolysis was evident.

Fig. 5(*c*) shows that, with fructose as substrate and with insulin (20 munits/ml), low concentrations of adrenaline did not increase PDH_a activity or decrease non-esterified fatty acid release as was found with glucose + insulin (Fig. 5*a*). On the other hand PDH_a activity was decreased in the presence of 0.1 μM-, 0.3 μM-and 1 μM-adrenaline ($P<0.01$, <0.001 and <0.01 for 0.1 μM, 0.3 μM and 1 μM respectively). Increased lipolysis was also observed at these concentrations of adrenaline, suggesting that the decrease in PDH_a activity may be secondary to the latter process. PDH_a activity in adipocytes incubated with fructose (with or without insulin) is very sensitive to increases in the concentration of non-esterified fatty acid (Sooranna & Saggerson, 1976*b*; and also see Fig. 6). This clearly does not appear to be the case in cells incubated with glucose (Fig. 5*b*).

The experiment summarized in Table 4 and Fig. 6 was devised to attempt to answer two questions. First, in the absence of insulin, can adrenaline decrease PDH_a activity when fructose is the carbohydrate substrate? Secondly, if PDH_a activity was decreased by adrenaline under these conditions would this decrease correlate with non-esterified fatty acid accumulation and could the effect of adrenaline be mimicked by addition of palmitate? Adrenaline concentrations were confined to 50 nM and above, i.e. to a concentration range where appreciable lipolysis may be expected. Nine individual experiments were performed in all, each of which had one control incubation, four incubations with additions of palmitate and five with additions of adrenaline. However, although non-esterified fatty acid release was increased by adrenaline in a dose-dependent fashion in every experiment, the scale of this net accumulation of fatty acids was extremely variable between experiments. The experimental approach was varied somewhat from that adopted for Figs. 5(*a*)–5(*c*). In essence all experiments contained an incubation flask to which 0.5 μM-adrenaline was added and another containing

Table 4. *Comparison of effects of* 0.5 μM-*adrenaline and various concentrations of palmitate on* PDH_a *activity in adipocytes incubated with fructose*

The data are taken from the experiment summarized in Fig. 6. Also, see the text for further details. The results are means ± S.E.M. *, ***, **** indicate $P < 0.05$, $P < 0.01$, $P < 0.001$ respectively for comparison of incubations containing adrenaline or palmitate versus the paired control. †, ††, †††, †††† indicate $P < 0.05$, $P < 0.02$, $P < 0.01$, $P < 0.001$ respectively for comparison of incubations containing adrenaline against paired ones containing palmitate.

Addition to incubation	PDH_a/glutamate dehydrogenase activity ratio	Non-esterified fatty acid concentration in medium at end of incubation (mM)	No. of paired determinations
None	0.264 ± 0.025	0.08 ± 0.01	6
Adrenaline (0.5 μM)	0.174 ± 0.030***	1.44 ± 0.54*	
Palmitate (0.35 mM)	0.131 ± 0.021****††	0.23 ± 0.02****	
None	0.261 ± 0.018	0.07 ± 0.01	9
Adrenaline (0.5 μM)	0.164 ± 0.020****	1.54 ± 0.42***	
Palmitate (0.5 mM)	0.107 ± 0.010****†††	0.38 ± 0.03****††	
None	0.243 ± 0.028	0.05 ± 0.01	4
Adrenaline (0.5 μM)	0.135 ± 0.023****	2.21 ± 0.55*	
Palmitate (1.0 mM)	0.098 ± 0.015***††	0.83 ± 0.02****	
Palmitate (1.75 mM)	0.106 ± 0.015***†	1.69 ± 0.07	
Palmitate (2.5 mM)	0.101 ± 0.018****†††	2.64 ± 0.05****	

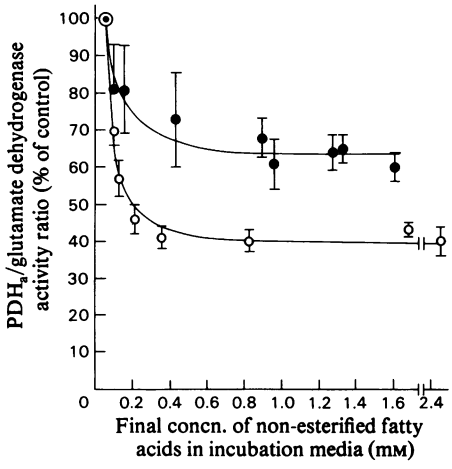

Fig. 6. *Relationship between PDH_a activity and final concentrations of non-esterified fatty acids in incubations of adipocytes with fructose*

Adipocytes were incubated as described in the Materials and Methods section with 5mM-fructose and either adrenaline (●) or sodium palmitate (○). Reading from left to right adrenaline additions were zero, 50nM, 0.15μM, 0.1μM, 0.3μM, 0.2μM, 0.9μM, 0.7μM, 0.5μM, and palmitate additions were zero, 0.15mM, 0.25mM, 0.35mM, 0.5mM, 1mM, 1.75mM, 2.5mM. The bars represent S.E.M. Further details of this experiment are given in the text. The mean fat-cell DNA was 7.0μg/ml of flask contents.

0.5mM-palmitate. In the other flasks the concentrations of adrenaline and palmitate were varied such that the total assembly of data is made up of incubations with: no additions (9); adrenaline, 50nM (5), 0.1μM (5), 0.15μM (4), 0.2μM (6), 0.3μM (8), 0.5μM (9), 0.7μM (4), 0.9μM (4); palmitate, 0.15mM (4), 0.25mM (5), 0.35mM (6), 0.5mM (9), 1.0mM (4), 1.75mM (4), 2.5mM (4), with the numbers of experiments given in parentheses. The values obtained for PDH_a activity were normalized by expression as percentages of the controls. The relationship between these and the final concentrations of non-esterified fatty acids in the incubation media are shown in Fig. 6. As shown previously (Sooranna & Saggerson, 1976*b*), when adipocytes were incubated with palmitate and fructose there was appreciable removal of fatty acid from the incubation medium. The hyperbolae fitted through these data were obtained from the regression lines relating the reciprocals of the values shown in Fig. 6 ($r = -0.955$ for the experiments with palmitate and $r = -0.928$ for the experiments with adrenaline). It is clear from Fig. 6 that, as in the presence of fructose and insulin (Fig. 5*c*), adrenaline decreased PDH_a activity in the presence of fructose alone, confirming the findings of Coore *et al.* (1971) with isolated adipocytes. Although non-esterified fatty acids were accumulated to concentrations of 1.8mM in the presence of glucose and 1μM-adrenaline (calculated from the data of Fig. 5*b*) there was no decrease in PDH_a activity under these conditions, suggesting that in the absence of insulin glucose, but not fructose, in some way protected against changes in PDH_a activity caused by adrenaline. Decreases in PDH_a activity caused by adrenaline (Fig. 6) were significant at and above 0.2μM-adrenaline ($P < 0.01$, <0.001, <0.001, <0.01 and <0.01 for 0.2μM, 0.3μM, 0.5μM, 0.7μM and 0.9μM respectively). However, the maximum decrease with palmitate was approx. 61% whereas with adrenaline the maximum decrease in PDH_a activity was only approx. 36%, even though fatty acid accumulation with adrenaline was comparable with the concentration of fatty acid remaining after incubation with high concentrations of palmitate. Table 4 states this statistically. Incubation with 0.5μM-adrenaline gave the greatest decrease in PDH_a activity (Fig. 6) and very considerable non-esterified fatty acid accumulation, yet PDH_a activity in the presence of 0.5μM-adrenaline was significantly greater than that in paired incubations with 0.35–2.5mM-palmitate. If the decreases in PDH_a activity observed with adrenaline (Fig. 6) were secondary to lipolysis it is concluded either that palmitate is more effective in decreasing PDH_a activity than the spectrum of fatty acids released by lipolysis or possibly that some other action of adrenaline opposes the decrease in PDH_a activity accompanying fatty acid accumulation, resulting in a smaller net decrease in PDH_a activity than that seen with fatty acids alone.

General conclusions

Changes in PDH_a activity correlated loosely with changes in fatty acid synthesis, suggesting that PDH may act as a point of 'coarse control' for this process. Incubated fat-pads have certain disadvantages compared with adipocytes for investigation of catecholamine control over PDH_a activity. With albumin present and with a sugar substrate that can facilitate fatty acid re-esterification, several situations can be observed in which adrenaline alters PDH_a activity in a manner that correlates with changes in lipolysis and non-esterified fatty acid accumulation. There are some unexplained differences in response to adrenaline between cells incubated with glucose and fructose, which are the subject of further investigation.

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