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Effects of streptozotocin-diabetes and insulin administration *in vivo* or *in vitro* on the activities of five enzymes in the adipose-tissue triacylglycerol-synthesis pathway

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1. At 2 days after administration of streptozotocin (100 mg/kg), activities in rat epididymal fat-pads of the following enzymes were significantly decreased: fatty acyl-CoA synthetase (FAS), mitochondrial and microsomal forms of glycerolphosphate acyltransferase (GPAT), monoacylglycerolphosphate acyltransferase (MGPAT) and Mg^{2+}-dependent phosphatidate phosphohydrolase (PPH). There were no significant changes in diacylglycerol acyltransferase or Mg^{2+}-independent PPH. 2. Insulin administration to diabetic rats over 2 days restored activities of FAS, both forms of GPAT, MGPAT and Mg^{2+}-dependent PPH. Significant restoration of all five activities was also seen 2 h after a single administration of insulin, but was not observed 45 min after insulin treatment. 3. Insulin significantly increased all five enzyme activities when adipocytes from diabetic rats were incubated for 2 h with a mixture of glucose, lactate, pyruvate and amino acids.

INTRODUCTION

Previously (Taylor & Saggerson, 1986) it was shown that streptozotocin-diabetes caused decreases in the specific activities of GPAT and Mg^{2+}-dependent PPH in rat adipose tissue. Reversal of these changes could be achieved by administration of insulin over 2 days or, to a certain extent, 2 h after a single administration of insulin to diabetic rats. These investigations are extended in the present study to consider the effects of diabetes and short- and long-term insulin administration on a wider range of enzymes in the triacylglycerol-synthesis pathway, namely FAS, MGPAT, DGAT and the Mg^{2+}-independent and Mg^{2+}-dependent PPHs and the mitochondrial and microsomal forms of GPAT. We have also investigated the effect of insulin *in vitro* on these activities when adipocytes isolated from diabetic rats are exposed to the hormone.

MATERIALS AND METHODS

Chemicals

These were obtained and treated as described by Bates & Saggerson (1979), Saggerson *et al.* (1980), Hall & Saggerson (1985) and Taylor & Saggerson (1986).

Animals

These were as described by Taylor & Saggerson (1986). Rats were taken as diabetic 2 days after a single subcutaneous injection of streptozotocin (100 mg/kg) dissolved in 0.2 ml of 50 mM-sodium citrate buffer (pH 4.0) containing 0.15 M-NaCl. All diabetic animals showed a strongly positive 'Clinistix' test for urinary glucose [> 0.5% (w/v) glucose]. Protamine zinc bovine insulin (Weddel Pharmaceuticals, London E.C.1, U.K.) was administered subcutaneously at 20 units/kg body wt. dispersed in 0.2 ml of 0.15 M-NaCl. Insulin injection was at approx. 10:00 h and, for the 2-day-insulin-treated animals, the injection was repeated after 24 and 48 h, and the rats were killed 2 h after the final injection. Saline-treated rats received a single subcutaneous injection of 0.2 ml of 0.15 M-NaCl.

Isolation and incubation of adipocytes

These were obtained from the pooled epididymal fat-pads of five diabetic rats by collagenase treatment (Rodbell, 1964) and washed twice with Krebs–Ringer bicarbonate medium (Krebs & Henseleit, 1932) containing fatty-acid-poor albumin (10 mg/ml). The cells were then suspended as a stock in 14 ml of the same medium.

Samples (3.0 ml) of the stock cell suspension were then added to each of four 50 ml silicone-treated flasks and incubated for 2 h with shaking (70 cycles/min) at 37 °C in a final volume of 10 ml of Krebs–Ringer bicarbonate containing fatty-acid-poor albumin (20 mg/ml), 5 mM-glucose, 1 mM-sodium lactate, 0.1 mM-sodium pyruvate and L-amino acids at the concentrations normally found in rat plasma (Mowbray & Last, 1974; Sooranna & Saggerson, 1979). The flask contents were continuously gassed with O_2/CO_2 (19:1). Two of the four flasks also received insulin (1 munit/ml), and the other two served as controls.

Preparation of homogenates

Rats were killed by cervical dislocation and both epididymal fat-pads were rapidly removed, cooled in ice-cold sucrose medium [0.25 M-sucrose/10 mM-Tris/HCl buffer (pH 7.4)/1 mM-EDTA/1 mM-dithiothreitol], weighed and immediately homogenized in 10 ml of ice-cold sucrose medium in a glass Potter–Elvehjem homogenizer with a Teflon pestle with a radial clearance of 0.2 mm. Homogenates were centrifuged at 2–4 °C by accelerating to 3000 $g_{av.}$, maintaining that field for 1 min and then decelerating with the brake on (integrated

Abbreviations used: DGAT, diacylglycerol acyltransferase (EC 2.3.1.20); FAS, ATP-dependent long-chain fatty acyl-CoA synthetase (EC 6.2.1.3); GPAT, glycerolphosphate acyltransferase (EC 2.3.1.15); LDH, lactate dehydrogenase (EC 1.1.1.27); MGPAT, monoacylglycerolphosphate acyltransferase (EC 2.3.1.51); NEM, *N*-ethylmaleimide; PPH, phosphatidate phosphohydrolase (EC 3.1.3.4).

Table 1. Effects of diabetes and insulin administration *in vivo* on enzyme activities in epididymal fat-pads

Animals were treated and tissue extracts prepared as described in the Materials and methods section. Bracketed body weights indicate those at start of streptozotocin treatment, time of insulin administration and time of death as appropriate. Bracketing of insulin- or saline-treated groups indicates that these treatments were performed at the same time and that significance of effects of insulin at 45 min and 2 h is assessed by comparison within these groups. The values are means ± S.E.M. for the numbers of preparations shown in parentheses: $^{a}P < 0.02$, $^{b}P < 0.01$, $^{c}P < 0.001$ for comparison with the control group (unpaired test); $^{d}P < 0.01$, $^{e}P < 0.001$ for comparison of insulin-treated groups with the appropriate untreated diabetic group (unpaired test).

Condition	Body wt. (g)	Wt. of pair of fat-pads (mg)	Protein content of fat-pads (mg per pair)	Enzyme specific activity (nmol/min per mg of protein)						Plasma glucose concn. (mM)
				FAS	Mitochondrial GPAT	Microsomal GPAT	MGPAT	Mg^{2+}-independent PPH	Mg^{2+}-dependent PPH	DGAT	
Control (10)	174±2	539±19	11.1±0.7	204±16	0.85±0.08	8.0±0.3	72.8±5.3	1.54±0.25	15.24±0.58	1.47±0.31	8.53±0.08
Diabetic (10)	164±2 152±3	316±21[c]	10.6±0.3	132±12[b]	0.34±0.04[e]	5.4±0.4[e]	43.3±2.4[e]	1.19±0.15	9.52±0.58[e]	1.04±0.12	28.87±1.25[e]
2-day-insulin-treated diabetic (6)	165±1 151±3 174±3	507±56[d]	15.3±1.2[bd]	186±8[d]	1.20±0.10[ae]	9.3±0.3[be]	93.8±4.0[be]	1.69±0.27	21.3±1.8[be]	0.90±0.12	4.20±0.10[ee]
45 min-saline-treated diabetic (6)	168±2 155±3	327±39[e]	11.2±0.5	106±7[e]	0.31±0.05[e]	5.6±0.3[e]	41.1±2.8[e]	1.22±0.11	10.23±0.66[e]	-	27.85±0.92[e]
45 min-insulin-treated diabetic (6)	166±3 152±3	298±35[e]	9.9±0.4	103±5[e]	0.23±0.03[e]	4.9±0.3[e]	38.5±1.9[e]	1.34±0.12	10.54±0.63[e]	-	21.32±1.30[ed]
2 h-saline-treated diabetic (10)	169±2 155±3	272±28[e]	10.3±0.7	128±6[e]	0.32±0.03[e]	5.3±0.4[e]	37.8±2.6[e]	1.48±0.18	9.32±0.63[e]	-	29.17±1.50[e]
2 h-insulin-treated diabetic (10)	171±3 156±3	271±23[e]	9.9±0.5	197±16[e]	0.50±0.03	7.0±0.4[d]	51.3±3.8[bd]	1.53±0.11	12.63±0.76[ad]	-	3.87±0.19[ee]

field-time = 4500 $g \cdot$min). The resulting infranatant was separated from the floating fat plug and the pellet and stored at −40 °C until used for assays.

Adipocytes were recovered from 10 ml incubations by centrifugation for 20 s at 200 $g_{av.}$, and the underlying medium was removed by aspiration. The cells were mixed with 4 ml of ice-cold sucrose medium and then homogenized and treated similarily to the fat-pad extracts.

Analytical methods

DGAT was assayed radiochemically at 30 °C (Coleman & Bell, 1976) by measurement of the dioleoylglycerol-dependent incorporation of [^{14}C]palmitoyl-CoA into hexane-soluble products. FAS was assayed fluorimetrically at 25 °C (Hall & Saggerson, 1985) by measurement of the incorporation of (1,N^6-etheno)-CoASH into the acid-precipitable reaction product palmitoyl-(etheno-CoA). GPAT was assayed radiochemically at 30 °C (Saggerson *et al.*, 1980; Rider & Saggerson, 1983) by measuring the incorporation of 1 mM-[U-^{14}C]glycerol 3-phosphate into butanol-soluble products. The mitochondrial form of GPAT, which is insensitive to NEM, was assayed in the presence of 10 mM-NEM by using 40 μM-palmitoyl-CoA to optimize conditions (Rider & Saggerson, 1983) The microsomal form of GPAT was assayed as NEM-sensitive activity (Saggerson *et al.*, 1979, 1980) and was optimized by use of 100 μM-palmitoyl-CoA (Rider & Saggerson, 1983). LDH was assayed spectrophotometrically at 25 °C and 340 nm (Saggerson, 1974). MGPAT was assayed spectrophotometrically at 25 °C and 412 nm (Saggerson *et al.*, 1980) by observing the transfer of CoA from oleoyl-CoA to 5,5′-dithiobis-(2-nitrobenzoic acid) (Okuyama *et al.*, 1971). PPH was assayed at 37 °C by measurement of phosphate release from an aqueous dispersion of phosphatidate (Taylor & Saggerson, 1986). Mg^{2+}-dependent PPH activity was calculated by subtraction of Mg^{2+}-dependent PPH from the total activity, which was measured in the presence of 2.5 mM-$MgCl_2$. Protein was measured by the method of Lowry *et al.* (1951), with fatty-acid-poor bovine albumin as a standard. Glucose in plasma was measured enzymically (Slein, 1963).

Statistical methods

Statistical significance was assessed by Student's *t* test for unpaired or paired samples as appropriate.

RESULTS AND DISCUSSION

Effects of diabetes and insulin administration *in vivo*

Table 1 shows specific activities of enzymes measured in homogenates from whole fat-pads. Combined mitochondrial + microsomal activities of GPAT were comparable with the total activity reported by Taylor & Saggerson (1986), but values for Mg^{2+}-dependent PPH activity were approximately half of those reported by Taylor & Saggerson (1986). The reason for this difference is unclear. Table 1 confirms Taylor & Saggerson (1986) in showing that total GPAT activity and Mg^{2+}-dependent PPH activity were decreased in diabetes and restored by insulin administration. We are unaware of any previous studies of adipose-tissue FAS, MGPAT or DGAT in diabetes, and Table 1 shows that diabetes resulted in significant decreases in the activities

of FAS (39%) and MGPAT (44%). By contrast, DGAT activity was not significantly changed. Table 1 shows that the previously reported decrease in total GPAT activity in diabetes (Taylor & Saggerson, 1986) is registered in both the microsomal form (32%) and the small mitochondrial activity (62%), whereas the significant 37% decrease in Mg^{2+}-dependent PPH activity is not accompanied by any significant change in the small Mg^{2+}-independent activity. Streptozotocin-treated animals did not have decreased food intake (hyperphagia in insulin-dependent diabetes is common), and had at least normal quantities of stomach and intestinal contents at death. Therefore the effects are attributed to the diabetic state rather than to a possible decrease in food intake owing to streptozotocin toxicity. Insulin administration to diabetic rats over 2 days resulted in activities that were significantly higher than the control values for both forms of GPAT, MGPAT and Mg^{2+}-dependent PPH. For FAS, insulin administration restored activity to the control value, but did not produce a supranormal activity. It is noteworthy that the amplitude of the change seen in the mitochondrial form of GPAT was larger than that for the microsomal enzyme, i.e. there was a 3.5-fold increase in mitochondrial GPAT on treatment of diabetic rats with insulin for 2 days, whereas microsomal GPAT only showed a 1.7-fold increase. This is perhaps analogous to the situation in the liver, where the mitochondrial form of GPAT appears to be particularly sensitive to changes in insulin status (Bates & Saggerson, 1977, 1979; Bates *et al.*, 1977). Diabetes significantly decreased the weight of the epididymal adipose tissue, but did not significantly alter the protein content. By contrast, the restoration of the tissue weight during 2 days of subsequent insulin treatment was accompanied by an increase on the tissue protein content. As a consequence, if the values shown in Table 1 had been expressed per pair of fat-pads rather than relative to tissue protein, the changes due to long-term insulin replacement would have been accentuated.

The bottom part of Table 1 demonstrates changes due to short-term insulin replacement. In these experiments insulin-treated diabetic rats were matched with saline-treated animals that were injected at the same time. As it happened, any trauma due to this procedure had no effect, relative to the non-injected diabetic group, on any of the measured enzyme activities or on the plasma glucose concentration. Table 1 shows that 2 h after administration of a single relatively large insulin dose to diabetic animals there were significant increases in activities of FAS, both forms of GPAT, MGPAT and Mg^{2+}-dependent PPH. For FAS and microsomal GPAT these restored activities were already not significantly different from the controls, whereas mitochondrial GPAT, MGPAT and Mg^{2+}-dependent PPH activities, although significantly elevated, were still below control values. An attempt to observe effects of insulin on the enzyme activities only 45 min after injection of the hormone to diabetic rats, however, proved unsuccessful. No activity was appreciably altered over this time, although plasma glucose concentrations were significantly decreased from approx. 28 mM to 21 mM (Table 1). This last observation indicates that insulin administered via the subcutaneous route should be able to exert acute effects on the tissue within this time scale. Another unquantified observation also suggested that insulin already had direct or indirect effects on the tissue over this time, in that the characteristic redness of epididymal fat-pads seen in diabetic rats was invariably lessened in the 45 min- or 2 h-insulin-treated groups. This is due to a decrease in blood flow into the tissue (E. D. Saggerson, unpublished work).

Effects of insulin *in vitro*

It was reasoned that effects of insulin on these enzyme activities in the diabetic state should be able to be reproduced *in vitro*. Accordingly, paired epididymal fat-pads from diabetic animals were incubated for 2 h with or without insulin (1 munit/ml), followed by homogenization of the tissue in the normal way. In four separate experiments insulin caused no significant or appreciable change in any of the enzyme activities. However, Table 2 shows that if adipocytes were first isolated from the fat-pads of diabetic rats and then incubated for 2 h, elevated activities of FAS, both forms of GPAT, MGPAT and Mg^{2+}-dependent PPH were observed relative to control incubations in cells treated with insulin. Values in these experiments were expressed relative to the LDH content of each cell homogenate, since the possibility of carry-over of albumin from the incubations caused difficulties in the estimation of the protein content of the cells. There was no difference between the LDH activities measured in control and insulin-treated cells.

General considerations

This study demonstrates that insulin can increase five enzyme activities associated with white-adipose-tissue glycerolipid synthesis when administered *in vivo* or *in vitro* after a period of diabetes. These changes would appear to be part of a co-ordinated response of the synthetic apparatus of the cell to the hormone. The lack of any apparent changes in DGAT activity is surprising. However, one is struck by the low activity of this enzyme relative to the others, and might question whether the DGAT assay is a true estimate. It is unlikely that the presentation of the diacylglycerol substrate to the enzyme is critical and may not be adequate in the assay. The present findings do not permit conclusions regarding the mechanisms involved. The time scales of the insulin effects *in vivo* and the presence of a full amino acid mixture in the cell incubations certainly do not preclude expression of long-term changes involving translation and transcription, as well as acute actions of the hormone. As far as we can ascertain, this work and that of Taylor & Saggerson (1986) represent the only studies of effects of insulin *in vivo* on these enzyme activities. On the other hand, certain acute effects of the hormone *in vitro* have been noted. Insulin has been reported to act on adipocytes from normal rats in the basal state to cause a slight elevation of total GPAT activity (Sooranna & Saggerson, 1976) and to increase FAS activity appreciably (Jason *et al.*, 1976), although the latter effect was not observed by others (Evans & Denton, 1977; Sooranna & Saggerson, 1978; Hall & Saggerson, 1985). In addition, insulin has been reported in the short term to have no effect on Mg^{2+}-dependent PPH activity in the basal state (Cheng & Saggerson, 1978), and there are no previous reports of any hormone effects on adipocyte MGPAT. By contrast, insulin rapidly re-activates adipocyte Mg^{2+}-dependent PPH, GPAT and FAS activities that have been previously lowered by exposure of adipocytes to catecholamines (Cheng & Saggerson, 1978; Rider &

Table 2. Effect on enzyme activities of incubation of adipocytes with insulin

Adipocytes isolated from diabetic rats were incubated for 2 h in duplicate with or without insulin (1 munit/ml) with other additions as indicated in the Materials and methods section. The values are means ± S.E.M. for six separate cell preparations: [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ (paired test).

Enzyme	Insulin in incubation	Activity (nmol/min per unit of LDH)	Change in activity by insulin	Percentage decrease in activity by insulin
FAS	−	295 ± 18	+52 ± 11[b]	19 ± 5[b]
	+	347 ± 19		
Mitochondrial GPAT	−	0.354 ± 0.034	+0.056 ± 0.020[a]	20 ± 7[a]
	+	0.410 ± 0.017		
Microsomal GPAT	−	8.48 ± 0.56	+1.86 ± 0.23[c]	22 ± 3[c]
	+	10.34 ± 0.65		
MGPAT	−	84 ± 8	+33 ± 9[b]	47 ± 19[a]
	+	117 ± 3		
Mg^{2+}-dependent PPH	−	20 ± 3	+4.6 ± 0.8[b]	25 ± 6[b]
	+	25 ± 3		

Saggerson, 1983; Hall & Saggerson, 1985). It is therefore possible that at least part of the insulin effects demonstrated here are acute effects and their demonstration may be facilitated by prior lowering of the activities by diabetes. It should, however, be pointed out that the present extraction conditions employ no precautions to facilitate the detection of acute hormone effects.

Further work should be directed towards understanding the effects of insulin shown here. The streptozotocin-diabetic rat would appear to offer a suitable system for studies *in vivo*, and the incubation of adipocytes obtained from diabetic rats could form the basis of a cell-culture system to study these phenomena.

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REFERENCES

Bates, E. J. & Saggerson, E. D. (1977) FEBS Lett. **84**, 229–232
Bates, E. J. & Saggerson, E. D. (1979) Biochem. J. **182**, 751–762
Bates, E. J., Topping, D.L., Sooranna, S. R., Saggerson, E. D. & Mayes, P. (1977) FEBS Lett. **84**, 225–228
Cheng, C. H. K. & Saggerson, E. D. (1978) FEBS Lett. **93**, 120–124
Coleman, R. & Bell, R. M. (1976) J. Biol. Chem. **251**, 4537–4543
Evans, G. L. & Denton, R. M. (1977) Biochem. Soc. Trans. **5**, 1288–1291
Hall, M. & Saggerson, E. D. (1985) Biochem. J. **226**, 275–282
Jason, C. J., Polokoff, M. A. & Bell, R. M. (1976) J. Biol. Chem. **251**, 1488–1492
Krebs, H. A. & Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. **210**, 33–66
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
Mowbray, J. & Last, K. S. (1974) Biochim. Biophys. Acta **349**, 114–122
Okuyama, H., Eibi, H. & Lands, W. E. M. (1971) Biochim. Biophys. Acta **248**, 263–273
Rider, M. H. & Saggerson, E. D. (1983) Biochem. J. **214**, 235–246
Rodbell, M. (1964) J. Biol. Chem. **239**, 275–280
Saggerson, E. D. (1974) Biochem. J. **140**, 211–224
Saggerson, E. D., Sooranna, S. R. & Cheng, C. H. K. (1979) INSERM Colloq. **87**, 223–238
Saggerson, E. D., Carpenter, C. A., Cheng, C. H. K. & Sooranna, S. R. (1980) Biochem. J. **190**, 183–189
Slein, M. W. (1963) in Methods of Enzymatic Analysis, 1st edn. (Bergmeyer, H. U., ed.), pp. 117–123, Academic Press, New York and London
Sooranna, S. R. & Saggerson, E. D. (1976) FEBS Lett. **64**, 36–39
Sooranna, S. R. & Saggerson, E. D. (1978) FEBS Lett. **92**, 241–244
Sooranna, S. R. & Saggerson, E. D. (1979) Biochem. J. **184**, 59–62
Taylor, S. J. & Saggerson, E. D. (1986) Biochem. J. **239**, 275–284

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