

# Factors influencing the altered thermogenic response of rat brown adipose tissue in streptozotocin-diabetes

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1. Adipocytes were isolated from the interscapular brown fat of male rats maintained at 21 °C. These animals were controls, streptozotocin-diabetics or 2-day insulin-treated diabetics. 2. With adipocytes from diabetic animals, maximum rates of noradrenaline-stimulated O<sub>2</sub> uptake were decreased by 58 %, and the  $B_{\max}$  of [<sup>3</sup>H]GDP binding to mitochondria was decreased by 55 %. Insulin administration reversed both of these changes. 3. Streptozotocin-diabetes increased basal lipolysis in adipocytes incubated with adenosine deaminase (1 unit/ml), decreased the EC<sub>50</sub> (concn. giving 50 % of maximum effect) for noradrenaline, but did not change the maximum rate of noradrenaline-stimulated lipolysis. Except for some small differences at very low concentrations (10–100 pM), diabetes or insulin treatment did not alter the sensitivity of noradrenaline-stimulated lipolysis or O<sub>2</sub> uptake to the inhibitory effect of N<sup>6</sup>-phenylisopropyladenosine. It is therefore concluded that the lesion(s) in thermogenesis in diabetes are not attributable to any changes in lipolysis. 4. Blood flow through interscapular brown fat, measured by accumulation of [<sup>14</sup>C]DDT [<sup>14</sup>C-labelled 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane] was increased by 2.3-fold 70 min after a single administration of insulin to diabetic rats. This treatment decreased blood flow through epididymal white fat by 58 %. 5. Propranolol treatment of diabetic rats muted the ability of insulin treatment to increase the maximum rate of noradrenaline-stimulated O<sub>2</sub> uptake, suggesting that this action of insulin may be a secondary one rather than a direct effect of the hormone on the adipocytes.

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## INTRODUCTION

It is now recognized in several animal models that brown adipose tissue plays an important role in cold-induced and diet-induced thermogenesis. The major stimulus for heat production appears to be noradrenaline released from the sympathetic innervation to the tissue. Noradrenaline binding to  $\beta$ -adrenoceptors leads to activation of adenylate cyclase, the cellular concentration of cyclic AMP is elevated, activating protein kinase and hence phosphorylating hormone-sensitive lipase, resulting in lipolysis. It has been suggested that the resulting increase in non-esterified fatty acids both activates a unique proton-conductance pathway to uncouple respiration from oxidative phosphorylation and becomes the substrate supply for the increased respiration (see reviews by Nedergaard & Lindberg, 1982; Nicholls & Locke, 1983, 1984; Cannon & Nedergaard, 1985*a,b*). This regulatable proton conductance is associated with 'thermogenin', a 32 kDa integral protein in the inner membrane of brown-adipocyte mitochondria, which also has the property of binding nucleotides such as GDP (Heaton *et al.*, 1978; Rial & Nicholls, 1983, 1984). Accompanying stimulation of thermogenesis within the brown adipocytes, there is a substantial increase in blood flow through the tissue (Heim & Hull, 1966; Foster & Frydman, 1979; Yahata *et al.*, 1983).

Streptozotocin-diabetic rats have decreased cold tolerance. In accord with this, basal and noradrenaline-stimulated heat production by rat interscapular brown fat is decreased in diabetes (Seydoux *et al.*, 1983), and diabetic rats do not exhibit an increase in metabolic rate

after consuming a palatable high-energy (cafeteria) diet (Rothwell & Stock, 1981). There are several possible sites for this diabetes-induced lesion in brown-fat thermogenesis. A defect somewhere in the mitochondrial  $\beta$ -oxidation pathway is inferred, since the carnitine-dependent conversion of [<sup>1-14</sup>C]palmitoyl-CoA into acid-soluble products by brown-adipose-tissue homogenates is decreased in diabetes (Seydoux *et al.*, 1983). The same laboratory (Seydoux *et al.*, 1984) has also reported a decrease in GDP binding to rat brown-fat mitochondria in diabetes, using an unspecified total concentration of [<sup>3</sup>H]GDP, suggesting that there is a change in the abundance, or GDP-binding properties, of thermogenin. A further possibility is that sympathetic-nerve activity in brown adipose tissue is decreased in diabetes, since insulin has been reported to stimulate sympathetic-nervous-system activity (Rowe *et al.*, 1981; Liang *et al.*, 1982; Rothwell *et al.*, 1983). Finally, the responsiveness to noradrenaline of the adenylate cyclase system and hence lipolysis in brown adipocytes might be altered in diabetes. It is now recognized in white adipocytes that receptor-mediated control of these processes is under dual regulation. Receptors for stimulatory agonists such as noradrenaline interact with adenylate cyclase through the G<sub>s</sub> coupling protein, whereas receptors for inhibitory paracrine agents such as adenosine or E-series prostaglandins are coupled through the G<sub>i</sub> protein. Dual regulation is also apparent in the brown adipocyte, because adenosine and prostaglandin E<sub>1</sub> inhibit noradrenaline-stimulated lipolysis and/or respiration (Szillat & Bukowiecki, 1983; Woodward & Saggerson, 1986) and because the presence of G<sub>i</sub> is inferred indirectly from

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Abbreviations used: DDT, 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane; EC<sub>50</sub>, the concentration of an agonist that causes 50 % of its maximum effect; PIA, N<sup>6</sup>-L-phenylisopropyladenosine.

the effects of *Bordetella pertussis* toxin (Woodward & Saggerson, 1986) and directly by immunological measurement (G. Milligan, J. A. Woodward & E. D. Saggerson, unpublished work). Any altered responsiveness of brown-adipocyte lipolysis to noradrenaline in diabetes could therefore result from a change in either the effectiveness of the stimulatory (noradrenaline) input or the competing inhibitory input to the system (e.g. through the  $A_1$  adenosine receptor). In this respect it is noteworthy that there are some parallels between the hypothyroid and the diabetic states, in that in hypothyroidism the thermogenic response of brown adipose tissue to electrical nerve stimulation or noradrenaline is also impaired (Mory *et al.*, 1981; Seydoux *et al.*, 1982). At the level of the brown adipocyte, hypothyroidism diminishes the maximum response of respiration and lipolysis to noradrenaline, increases the  $EC_{50}$  for noradrenaline and increases sensitivity to the anti-lipolytic action of PIA (Woodward & Saggerson, 1986). In addition, GDP binding to brown-adipose-tissue mitochondria from rats briefly exposed to 4 °C is decreased in hypothyroidism (Triandafillou *et al.*, 1982).

The primary objective of this study was therefore to investigate the responsiveness of lipolysis to noradrenaline and the  $A_1$ -adenosine-receptor agonist PIA, with brown adipocytes from diabetic or insulin-treated diabetic rats, and to relate any observed changes to alterations in the thermogenic response of the cells. The second objective was to investigate more fully the effect of diabetes (and insulin therapy) on the characteristics of GDP binding to brown-adipocyte mitochondria. Measurements of relative blood flow in brown and white fat from diabetic animals are also reported, showing effects of insulin which are relevant to the rest of the study.

## MATERIALS AND METHODS

### Chemicals

These were obtained and treated as described by Taylor & Saggerson (1986) or Woodward & Saggerson (1986). In addition [ $^{14}$ C]DDT, [ $^{14}$ C]sucrose and [ $^3$ H]GDP were obtained from Amersham International, and sodium pentobarbitone was a gift from Abbot Laboratories (Queenborough, Kent, U.K.). Before use of [ $^{14}$ C]DDT, hexane was evaporated under a stream of nitrogen, and the DDT was dissolved in 0.15 M-NaCl containing 2.5% (v/v) Tween 20.

### Animals

These were male Sprague-Dawley rats bred at University College, maintained at approx. 21 °C on a 13 h-light/11 h-dark cycle with light from 06:00 to 19:00 h. The rats were fed on Rat and Mouse No. 3 Breeding Diet (Special Diet Services, Witham, Essex, U.K.). Weight ranges of animals, induction of diabetes and treatment of diabetic animals with insulin was as described by Saggerson & Carpenter (1987).

### Isolation of brown adipocytes

The procedure originally described by Fain *et al.* (1967) and elaborated by Nedergaard & Lindberg (1982) was used, as described by Woodward & Saggerson (1986).

### Measurements of lipolysis and oxygen consumption by adipocytes

These were performed as described by Woodward & Saggerson (1986).

### Isolation of brown-adipocyte mitochondria

These were isolated from freshly prepared adipocytes by procedures identical with those used to obtain mitochondria from whole interscapular brown adipose tissue (Saggerson & Carpenter, 1982). Mitochondrial protein was initially standardized by a biuret assay (Bradford, 1976) and subsequently redetermined in stored mitochondrial samples by the method of Lowry *et al.* (1951), with bovine albumin as standard.

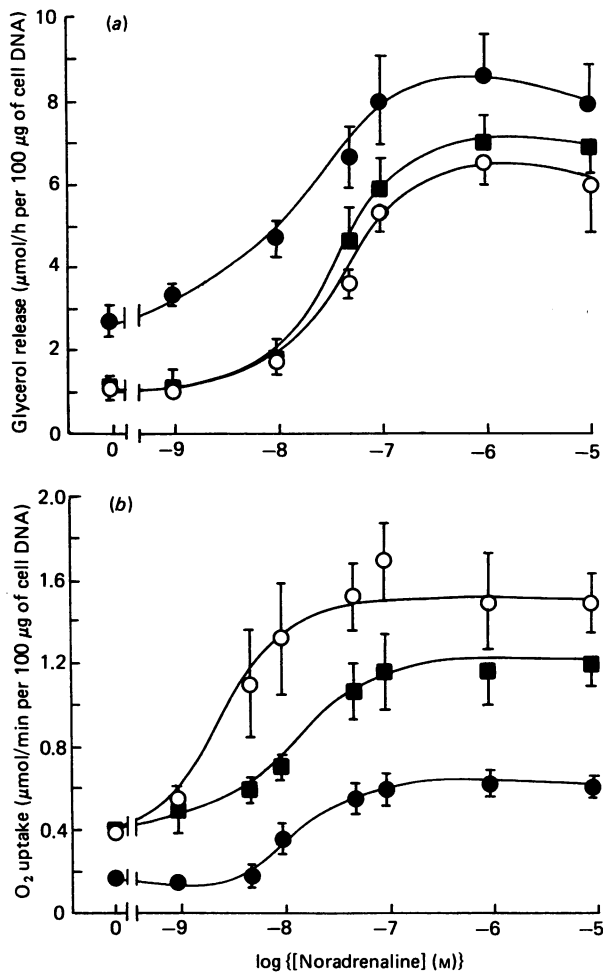
### Specific binding of GDP to brown-adipocyte mitochondria

This was essentially as described by Rial & Nicholls (1983), except that 'non-specific' binding was simultaneously measured in the presence of 1.0 mM unlabelled GDP. When these values were subtracted from total binding, 'specific binding' was apparent at only a single class of high-affinity sites. The binding assay was performed for 5 min at 30 °C in a total volume of 0.25 ml containing 5 mM-Tes (pH 7.0), 100 mM-KCl, fatty acid-poor albumin (20  $\mu$ M), rotenone (2.5  $\mu$ M), [ $^3$ H]GDP (0.3  $\mu$ Ci/ml), [ $^{14}$ C]sucrose (0.4  $\mu$ Ci/ml), mitochondria (0.3 mg/ml) and GDP (1–15  $\mu$ M). The assay was terminated by centrifugation for 2 min at 6500  $g_{av}$  in an Eppendorf 5412 centrifuge, and the supernatant was discarded. Excess fluid on the sides of the tubes was removed with tissue paper. The mitochondrial pellets were solubilized in 0.5 ml of 10% (v/v) Triton X-100 at 30 °C and taken for dual-label counting in 13 ml of scintillation fluid [80 g of naphthalene plus 4 g of 2,5-bis-(5-t-butylbenzoxazol-2-yl)thiophen in 600 ml of toluene plus 400 ml of 2-methoxyethanol]. The binding data were analysed in two ways. In method I, linear Scatchard plots for each experiment were used to determine  $B_{max}$  and  $K_D$ . These values were then measured. In method II, mean values  $\pm$  S.E.M. for specific binding at each GDP concentration were first determined (these values are shown in Fig. 4) and then used in a least-squares fitting computer program to obtain estimates of  $B_{max}$  and  $K_D$ .

### Measurement of adipose-tissue relative blood flow in diabetic rats

This method, originally described by Herd *et al.* (1968) and used more recently by Rofe & Williamson (1983), exploits the high lipid/aqueous partition coefficient (approx. 4000:1) of the insecticide DDT.

Experiments were commenced between 09:00 and 12:00 h. At zero time diabetic rats either received a subcutaneous injection of 0.2 ml of 0.15 M-NaCl or of protamine-zinc insulin (20 units/kg) dispersed in 0.2 ml of 0.15 M-NaCl. At 60 min the rats were anaesthetized by an intraperitoneal injection of sodium pentobarbitone (50 mg/kg) dissolved in approx. 0.2 ml of 0.15 M-NaCl. At 70 min 0.2 ml of [ $^{14}$ C]DDT solution (1.5  $\mu$ Ci) was injected into the heart, followed by sampling of blood from the tail at 71 and 80 min. At 81 min the rats were killed by injection of 0.4 ml of saturated KCl into the heart. Samples of tissue were removed, rinsed in 0.15 M-NaCl, blotted, weighed and homogenized (Ultra-Turrax) in 10 ml of propan-2-ol/hexane/1 M- $H_2SO_4$  (40:10:1, by



**Fig. 1. Dose/response curves for stimulation of brown-adipocyte lipolysis and respiration by noradrenaline in the presence of adenosine deaminase**

Cells were incubated in medium containing 5 mM-glucose, fatty acid-poor albumin (40 mg/ml), adenosine deaminase (1 unit/ml) and the indicated concentrations of noradrenaline. The values are means  $\pm$  S.E.M. (a) Lipolysis measurements (all  $n = 4$ ):  $\circ$ , control;  $\bullet$ , diabetic;  $\blacksquare$ , insulin-treated diabetic. (b)  $O_2$ -uptake measurements; symbols as for (a) ( $n = 4$  for diabetic and  $n = 5$  for other two states).

vol.) (Dole, 1956). After addition of 6 ml of hexane and 7 ml of water, 4 ml portions of the upper hexane layer were taken for liquid-scintillation counting. In addition, 3 ml portions of hexane layers from white- and brown-fat extractions were evaporated to dryness and weighed to determine the amount of extractable lipid.

Glucose was measured enzymically (Slein, 1963) in plasma obtained from the 80 min samples.

#### Measurement of adipocyte DNA

The DNA content of each brown-adipocyte preparation was determined fluorimetrically by the method of Switzer & Summer (1971).

#### Statistical methods and presentation of data

Values are shown in Figures as means  $\pm$  S.E.M. Where S.E.M. bars are not shown in Figures, these lie within the area of the symbol. Values of  $n$  in legends refer to the

**Table 1.  $EC_{50}$  values for stimulation of lipolysis and respiration by noradrenaline**

The values are calculated from the experiments shown in Figs. 1(a) and 1(b) and are means  $\pm$  S.E.M.: <sup>a</sup> $P < 0.02$ , <sup>b</sup> $P < 0.01$  for effects of adenosine deaminase; <sup>c</sup> $P < 0.01$ , <sup>d</sup> $P < 0.001$  for comparisons with the diabetic state. There were no significant differences between the control and the insulin-treated diabetic states.

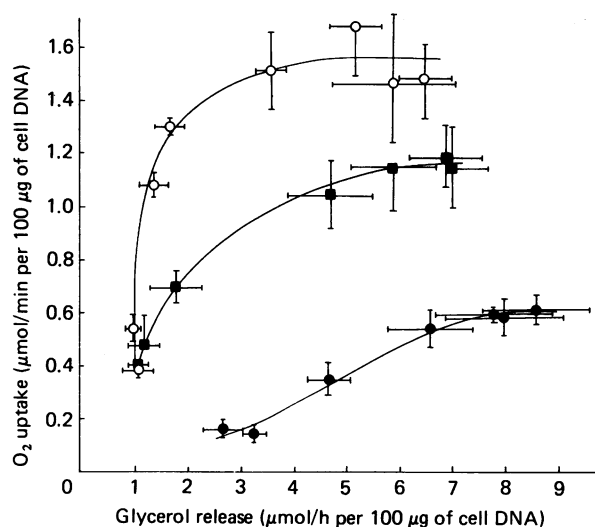
Condition	Adenosine deaminase (1 unit/ml)	$EC_{50}$ for noradrenaline (nM)	
		Lipolysis	$O_2$ uptake
Control	—	115 $\pm$ 9	13 $\pm$ 2
	+	55 $\pm$ 5 <sup>bd</sup>	8 $\pm$ 3
Diabetic	—	143 $\pm$ 41	39 $\pm$ 12
	+	14 $\pm$ 3 <sup>a</sup>	18 $\pm$ 6
Insulin-treated diabetic	—	112 $\pm$ 20	34 $\pm$ 10
	+	43 $\pm$ 4 <sup>ac</sup>	17 $\pm$ 5

numbers of separate cell preparations. Statistical significance was determined by Student's  $t$  test for paired or unpaired samples as appropriate.

## RESULTS AND DISCUSSION

### Effects of adenosine deaminase and noradrenaline on lipolysis and $O_2$ uptake

Fig. 1 shows dose/response curves for stimulation of these processes by noradrenaline. As found by Woodward & Saggerson (1986), addition of adenosine deaminase to remove endogenous adenosine from cell incubations caused a small but significant decrease in the  $EC_{50}$  for stimulation of lipolysis, this effect being more pronounced in the diabetic state (Table 1). Small non-significant decreases in the  $EC_{50}$  for stimulation of respiration were also observed with adenosine deaminase. Since the concentrations of the inhibitory agonist adenosine in cell incubation media might vary with physiological state, it was considered more appropriate to compare noradrenaline dose/response curves in the presence of adenosine deaminase (at the maximally effective concentration of 1 unit/ml; Woodward & Saggerson, 1986). Diabetes had no significant effect on the maximum noradrenaline-stimulated rate of lipolysis, but increased the basal rate by 2.7-fold ( $P < 0.02$ ; Fig. 1a) and decreased the  $EC_{50}$  for noradrenaline by 3-fold (Table 1). Insulin administration reversed both of these changes. By contrast, diabetes decreased the basal rate of respiration by 53% ( $P < 0.01$ ) and also decreased the maximum rate of noradrenaline-stimulated  $O_2$  uptake (arbitrarily taken at  $1 \mu M$ -noradrenaline) by 58% ( $P < 0.02$ ; Fig. 1b). Insulin administration restored the basal rate of respiration to that of the control, and significantly ( $P < 0.05$ ) increased the maximum rate of respiration to a value not significantly different from the control. Clearly, therefore, insulin deprivation causes a lesion in brown-adipocyte thermogenesis which cannot be attributed to any decrease in responsiveness of lipolysis to noradrenaline. By contrast, hypothyroidism, which also decreases basal and maximum rates of respiration



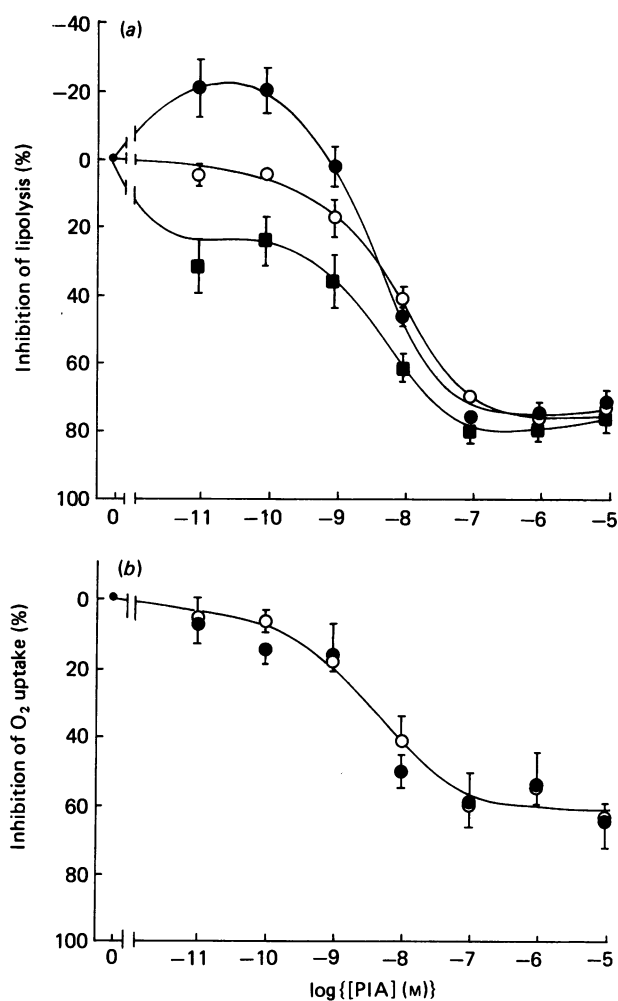
**Fig. 2. Relationship between brown-adipocyte respiration and lipolysis in the presence of adenosine deaminase.**

The values represent the data from Figs. 1(a) and 1(b) plotted against each other. Symbols as for Fig. 1.

by approx. 50%, is accompanied by a 57% decrease in the maximum rate of lipolysis (Woodward & Saggerson, 1986).

If all of the increased respiration on stimulation of the cells by noradrenaline is fuelled by oxidation of fatty acids released by lipolysis, then  $1 \mu\text{mol}$  of increased glycerol release/h ( $3 \mu\text{mol}$  of fatty acid) could support an increase in respiration of  $1.15 \mu\text{mol}$  of  $\text{O}_2/\text{min}$  ( $69 \mu\text{mol}$  of  $\text{O}_2/\text{h}$ ), assuming that none of this fatty acid was released from the cells and that none was re-esterified. Fig. 2 shows the relationship between respiration and lipolysis. In normal cells, an increase in lipolysis of  $1 \mu\text{mol}/\text{h}$  above the basal rate is accompanied by an increase in respiration of approx.  $1.05 \mu\text{mol}$  of  $\text{O}_2/\text{min}$ , i.e. quite close to the theoretical maximum. At higher rates of lipolysis, this steep relationship reaches a plateau, presumably because of saturation of the oxidation system. In the diabetic state, in addition to the decrease in the maximum rate of respiration, the relationship between respiration and lipolysis is less steep, suggesting the possibilities that a greater proportion of the available fatty acid is released from the cells, that the proton conductance pathway is less sensitive to activation by fatty acids, that lipolysis is relatively underestimated in the control state if glycerokinase activity were to be decreased in diabetes, or that a greater proportion of the fatty acid is re-esterified. This last possibility can be discounted, since fatty acid esterification by brown adipocytes from diabetic rats is decreased by 80% in diabetes (Baht & Saggerson, 1988).

In a parallel study with normal animals (E. D. Saggerson, unpublished work), the  $\text{EC}_{50}$  for noradrenaline stimulation of white-adipocyte lipolysis was determined in the presence of adenosine deaminase (1 unit/ml) and found to be  $4.2 \pm 0.3 \text{ nM}$  (mean  $\pm$  s.e.m. for six experiments). This may be contrasted with the value of  $55 \pm 5 \text{ nM}$  for brown adipocytes from age-matched normal rats (Table 1), and possibly suggests a difference in the adrenergic activation of lipolysis between the two cell types. In this respect it is noteworthy that Arch *et al.*



**Fig. 3. Inhibition of noradrenaline-stimulated brown-adipocyte lipolysis and respiration by PIA in the presence of adenosine deaminase**

Incubation conditions were as for Fig. 1, except that noradrenaline was present in each case at a concentration that causes 50% of its maximum effect ( $\text{EC}_{50}$ ) together with the indicated concentrations of PIA. Symbols as for Fig. 1. The values are means  $\pm$  s.e.m. and are expressed relative to those with noradrenaline alone. (a) Lipolysis measurements:  $n = 4, 3$  and  $5$  for control, diabetic and insulin-treated diabetic states respectively. (b)  $\text{O}_2$ -uptake measurements:  $n = 4$  and  $3$  for control and diabetic states respectively.

(1984) have reported the rat brown-adipocyte  $\beta$ -adrenoceptor as being atypical.

#### Effects of PIA on lipolysis and $\text{O}_2$ uptake

Fig. 3(a) shows that when lipolysis was stimulated by a 50% maximally effective concentration of noradrenaline, PIA inhibited the resulting lipolysis with an  $\text{EC}_{50}$  of approx.  $10 \text{ nM}$ . The same was also found with cells from diabetic animals. Surprisingly, very low concentrations of PIA stimulated lipolysis in cells from diabetic animals ( $P < 0.05$  by paired difference at  $10 \text{ pM}$ - and  $100 \text{ pM}$ -PIA). The reason for this is unclear, and it seems unlikely that this effect could be mediated through stimulatory  $\text{A}_2$ -type adenosine receptors, since PIA should have lower potency at these than at the inhibitory

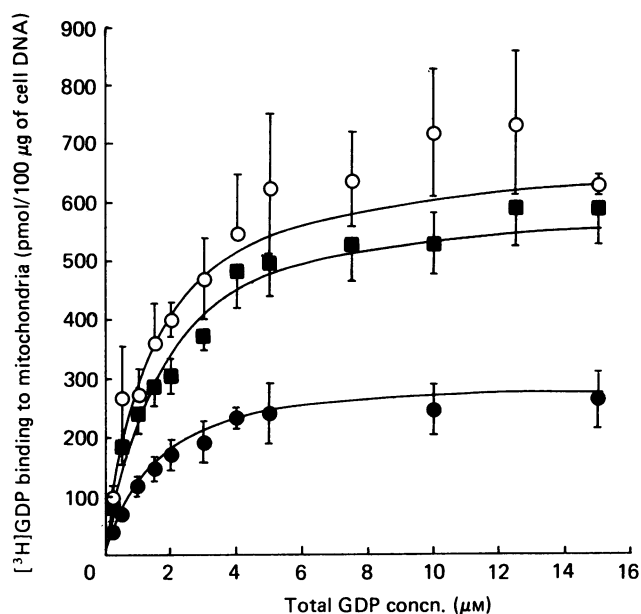


Fig. 4. Specific binding of [<sup>3</sup>H]GDP to brown-adipocyte mitochondria

Symbols as for Fig. 1. The values are means  $\pm$  S.E.M. for four experiments in each case. The lines are best fits selected by computer.  $B_{max}$  and  $K_D$  are shown in Table 2.

$A_1$ -type receptor. The PIA dose/response curve in the insulin-treated diabetic case was also complex, showing abolition by insulin treatment of the stimulatory effects of PIA. Complex PIA dose/response curves with white adipocytes in some instances have been reported by Saggerson (1986). Fig. 3(b) shows that very low concentrations of PIA did not stimulate respiration and that PIA inhibited up to 60% of this process with an  $EC_{50}$  of 3 nM in the normal and the diabetic states. The physiological significance, if any, of the effects of very low concentrations of PIA in diabetes are unclear, but Fig. 3 indicates that at concentrations in excess of 1 nM PIA diabetes has no appreciable effect on the responsiveness to this inhibitory agonist. The likelihood that adenosine effects are not involved in the lesion in

thermogenesis in diabetes is also reinforced by the observation that the activity of the ectoenzyme 5'-nucleotidase is not changed in brown adipocytes in this state (Jamal & Saggerson, 1987).

#### GDP binding to brown-adipocyte mitochondria

Isolating cells before preparation of mitochondria permits measurement of GDP binding without interference from non-adipocyte mitochondrial protein and permits direct comparisons of  $B_{max}$  values between physiological states on a cell-DNA basis. Citrate synthase activity was measured both in whole homogenates and in mitochondrial fractions, and was used as a reference enzyme to calculate the amount of mitochondrial protein per quantity of cell DNA and thereby to relate GDP binding to cell DNA. Either method of calculating binding (see the Materials and methods section) gave qualitatively similar results in that diabetes decreased the  $B_{max}$  of GDP binding relative to both mitochondrial protein and cell DNA and insulin treatment restored the value (Fig. 4 & Table 2). Changes in physiological state did not alter the  $K_D$  for GDP binding. The 55% decrease in the  $B_{max}$  relative to cell DNA correlates very closely with the scale of the decrease in respiration expressed on the same basis (Fig. 1b), as also does the degree of restoration by insulin. Although other factors may also be involved, it would seem likely that a direct or indirect action of insulin regulates the thermogenin content of the brown adipocyte, and thereby the rate of respiration. Seydoux *et al.* (1984) reported increased binding of an unspecified amount of GDP to rat interscapular brown-fat mitochondria from insulin-infused normal animals, and Jacobsson *et al.* (1986) have found, using a cDNA probe, that insulin injection into normal mice causes a small increase in the brown-adipose-tissue content of thermogenin mRNA. In addition, a decreased brown-adipose-tissue metabolic activity in starvation is accompanied by decreased GDP binding to mitochondria, and recovery of normal function on re-feeding with a carbohydrate meal appears to be associated with insulin secretion (Rothwell *et al.*, 1983, 1984). It is also noteworthy that decreased respiration in hypothyroidism (Woodward & Saggerson, 1986) is accompanied by a significant decrease in GDP binding to rat brown-adipocyte mitochondria (J. A. Woodward & E. D. Saggerson, unpublished work).

Table 2. Specific binding of [<sup>3</sup>H]GDP to brown-adipocyte mitochondria

Experimental procedures and methods for calculation of binding parameters are shown in the Materials and methods section. The values are means  $\pm$  S.E.M. from four separate adipocyte/mitochondrial preparations in each case, as shown in Fig. 4: <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.02$ , <sup>c</sup> $P < 0.01$ , <sup>d</sup> $P < 0.001$  for comparisons with the diabetic state. There were no significant differences between the control and the insulin-treated diabetic states.

Condition	GDP binding to mitochondria ( $B_{max}$ )				
	(pmol/mg of mitochondrial protein) (Method I)	(pmol/100 $\mu$ g of cell DNA)		$K_D$ ( $\mu$ M)	
		(Method I)	(Method II)	Method I	Method II
Control	417 $\pm$ 21 <sup>c</sup>	812 $\pm$ 75 <sup>c</sup>	689 $\pm$ 9 <sup>d</sup>	1.88 $\pm$ 0.22	1.30 $\pm$ 0.10
Diabetic	275 $\pm$ 28	366 $\pm$ 64	306 $\pm$ 11	2.03 $\pm$ 0.22	1.43 $\pm$ 0.09
Insulin-treated diabetic	395 $\pm$ 28 <sup>a</sup>	639 $\pm$ 52 <sup>b</sup>	613 $\pm$ 21 <sup>d</sup>	1.56 $\pm$ 0.19	1.56 $\pm$ 0.14

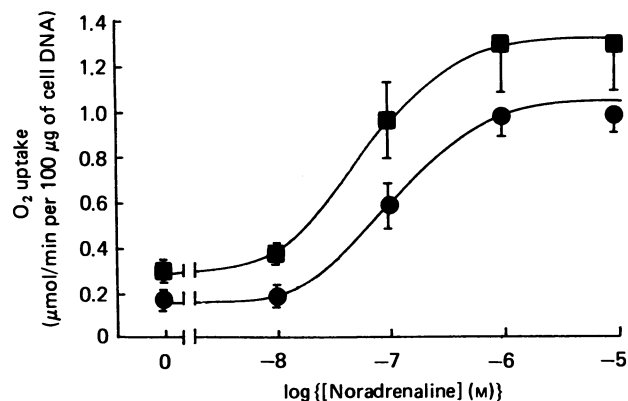
**Table 3. Effect of insulin injection on relative blood flow in adipose tissues of diabetic rats**

Full details are given in the Materials and methods section. The values are means  $\pm$  S.E.M. for seven rats in both cases. Statistically significant effects of insulin are indicated by: <sup>a</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$ ; <sup>c</sup> $P < 0.001$ .

Rats ...	Saline-treated	Insulin-treated
Body wt. (g)		
Before streptozotocin treatment	167 $\pm$ 4	170 $\pm$ 2
48 h after streptozotocin	157 $\pm$ 6	162 $\pm$ 3
Plasma glucose (mM)	23.5 $\pm$ 1.1	14.4 $\pm$ 1.5 <sup>c</sup>
Extractable lipid (% of tissue wet wt.)		
Epididymal white fat	55 $\pm$ 1	56 $\pm$ 1
Interscapular brown fat	26 $\pm$ 3	22 $\pm$ 2
10 <sup>-3</sup> $\times$ Relative blood flow (d.p.m./g wet wt.)		
White fat (W)	48.1 $\pm$ 7.8	21.8 $\pm$ 3.1 <sup>b</sup>
Brown fat (B)	21.5 $\pm$ 2.2	43.6 $\pm$ 6.6 <sup>b</sup>
Muscle (M)	7.4 $\pm$ 0.7	7.2 $\pm$ 0.8
Liver	123 $\pm$ 21	107 $\pm$ 18
Ratio B/W	0.55 $\pm$ 0.08	1.99 $\pm$ 0.14 <sup>c</sup>
Ratio B/M	3.24 $\pm$ 0.27	6.01 $\pm$ 0.59 <sup>b</sup>
Ratio W/M	6.29 $\pm$ 0.52	3.06 $\pm$ 0.30 <sup>c</sup>
10 <sup>-3</sup> $\times$ Relative blood flow (d.p.m./g of extractable lipid)		
White fat (W')	92.4 $\pm$ 14.4	38.6 $\pm$ 5.4 <sup>b</sup>
Brown fat (B')	97.2 $\pm$ 18.9	227 $\pm$ 49 <sup>a</sup>
Ratio B'/W'	1.06 $\pm$ 0.10	5.41 $\pm$ 0.68 <sup>c</sup>
10 <sup>-3</sup> $\times$ Radioactivity in blood (d.p.m./ml)		
1 min	29.8 $\pm$ 4.2	23.2 $\pm$ 4.8
10 min	9.9 $\pm$ 2.7	7.9 $\pm$ 1.3

#### Effect of insulin administration on relative blood flow in brown and white adipose tissues of diabetic rats

In general, alterations in brown-adipose-tissue metabolic activity are accompanied by parallel changes in blood flow through the tissue, as exemplified by acute and chronic cold exposure (Foster & Frydman, 1979; Foster *et al.*, 1980), where both parameters are increased, or by starvation, in which they are decreased (Rothwell *et al.*, 1983; Fernandez *et al.*, 1987). Although the [<sup>14</sup>C]DDT partition method used here (Table 3) is only relative and should not be used to estimate absolute rates of flow per g wet wt. of tissue, it would seem acceptable to compare data between brown and white adipose tissue when these are expressed relative to tissue lipid contents. As pointed out by Rofe & Williamson (1983), this method is particularly useful for comparative studies with adipose tissue, since it exploits the high affinity for DDT of neutral fat (Finnegan *et al.*, 1949; Herd *et al.*, 1968). Equilibrium between the lipid and aqueous phases is not reached for many hours and thus provides an average measure of blood flow over longer periods of time than can be used with other indirect methods. Table 3 shows that, expressed relative to tissue lipid content, DDT accumulations in interscapular brown fat and epididymal white fat were approximately the same in saline-treated diabetic animals. By contrast, DDT accumu-

**Fig. 5. Dose/response curves for stimulation of respiration in adipocytes from propranolol-treated rats**

Animals were treated with propranolol (10 mg/kg subcutaneously) on the day of streptozotocin administration and daily thereafter. Incubation conditions were as for Fig. 1. The values are means  $\pm$  S.E.M. ( $n = 4$ ); ●, diabetic; ■, insulin-treated diabetic.

lation relative to tissue lipid was 8-fold higher in brown than in white fat in 24 h-starved rats of substantially larger (240–300 g) body wt. than used here (Rofe & Williamson, 1983). Table 3 shows that, 70 min after a single administration of insulin, blood flow was increased 2.3-fold in brown adipose tissue and decreased by 58% in white fat, so that the DDT accumulation in brown fat then exceeded that in white fat by 5-fold. Although blood glucose concentration declined after insulin administration, the rats were not hypoglycaemic during the period of blood-flow measurement. The implications of this experiment are that insulin deprivation decreases blood flow through brown adipose tissue, paralleling the decreased thermogenic capability of the brown adipocytes. We are unaware of any previous reports of acute regulation of brown-adipose-tissue blood flow by insulin, especially in diabetic animals. However, in white adipose tissue Madsen & Malchow-Møller (1983) observed decreases in blood flow after glucose infusion into fed rats, and suggested that this change was secondary to a rise in plasma insulin concentration.

Insulin is reported to exert a direct stimulatory action on the central nervous system independent of hypoglycaemia in the rat (Rothwell *et al.*, 1983), in the dog (Liang *et al.*, 1982) and in man (Rowe *et al.*, 1981), and therefore it is possible that the effects of insulin, both to restore brown-adipocyte thermogenesis and to increase blood flow through the tissue in the diabetic rat, are secondary to restoration of normal long-term sympathetic-nerve stimulation of the tissue, rather than being direct actions of the hormone on the tissue. It is noteworthy that insulin has been implicated in the restoration of a normal thermogenic response on re-feeding of starved rats and that increased metabolic rate on treatment of starved rats with insulin can be blocked by propranolol (Rothwell *et al.*, 1983). Lastly, it is also pertinent that adrenaline infusion into starved rats respectively increases and decreases DDT accumulation in interscapular brown fat and epididymal white fat (Rofe & Williamson, 1983), thus paralleling the effects of insulin shown here.

**Effect of insulin in propranolol-treated rats**

With cells from propranolol-treated diabetic rats, basal rates of O<sub>2</sub> uptake (Fig. 5) were similar to those in untreated diabetic rats (Fig. 1*b*). Insulin administration to propranolol-treated diabetic rats increased basal O<sub>2</sub> uptake by 82% ( $P < 0.01$ ), but only to rates which were still significantly lower ( $P < 0.02$ ) than those seen in cells from insulin-treated animals without propranolol. By contrast, rates of O<sub>2</sub> uptake at 0.1 μM-, 1 μM- and 10 μM-noradrenaline were not significantly different in cells from propranolol-treated diabetic animals with or without insulin therapy (Fig. 5). Maximum noradrenaline-stimulated rates of O<sub>2</sub> uptake were significantly higher ( $P < 0.02$ ) in cells from propranolol-treated diabetic animals (Fig. 5) than in untreated diabetic rats (Fig. 1*b*), and the EC<sub>50</sub> for noradrenaline of approx. 100 nM was also appreciably higher (see Table 1). It is not clear whether these changes are due solely to propranolol administration or reflect the performance of the experiments in Figs. 1*b* and 5 at different times. After subtraction of basal rates, the dose/response curves shown in Fig. 5 are almost superimposable, with insulin increasing the maximum effect of noradrenaline (the rate at 1 μM-noradrenaline minus the basal rate) by only 1.2-fold. By contrast, without propranolol treatment, insulin administration had a larger effect, increasing the maximum effect of noradrenaline by 1.7-fold (calculated from Fig. 1*b*). Propranolol treatment therefore blocked much of the effect of insulin to enhance the thermogenic response in diabetes, suggesting that the effect of insulin is a secondary one and is not a direct action of the hormone at the level of the adipocytes.

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