Effect of corticotropin on brown adipose tissue mitochondrial GDP binding in obese rats

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Corticotropin stimulated brown adipose tissue mitochondrial GDP binding of young obese rats to the levels seen in lean rats. This effect was attenuated by chronic increases in corticosterone. The stimulatory response to corticotropin was absent from lean rats unless endogenous secretion of corticosterone was prevented.

The obesity of Zucker *fa/fa* rats has been associated with the loss of brown adipose tissue thermogenic responses to dietary signals (Rothwell *et al.*, 1981; Holt *et al.*, 1983) resulting in an increase in energetic efficiency (Marchington *et al.*, 1983). This defect is thought to reflect the loss of diet-induced sympathetic stimulation of brown adipose tissue (D. Marchington & D. A. York, unpublished work) rather than a deficit in brown adipose tissue itself, which shows a normal increase in thermogenic capacity when young obese rats are exposed to cold environments (Holt *et al.*, 1983; Triandafillou & Himms-Hagen, 1983). However, the development of obesity in the *fa/fa* rat is prevented by adrenalectomy, which results in restoration of brown adipose tissue responses to dietary signals (Holt & York, 1982; Holt *et al.*, 1983; Marchington *et al.*, 1983) associated with the normalization of sympathetic activity in brown adipose tissue (D. Marchington & D. A. York, unpublished work). The specific binding of GDP, an inhibitor of the thermogenic proton translocation, to mitochondria has been used extensively as a measure of the thermogenic capacity of brown adipose tissue mitochondria (Rothwell *et al.*, 1982). Corticosterone has been shown to suppress the normal increase in brown adipose tissue mitochondrial GDP binding after feeding cafeteria diets, but has no effect on the increase in binding after cold acclimation (Galpin *et al.*, 1983). These experiments suggested that corticosterone may regulate the response of brown adipose tissue mitochondria to dietary signals. However, since induced changes in circulating corticosterone levels in these experiments would be associated with reciprocal changes in corticotropin it was not clear which hormone was primarily responsible for the changes in brown adipose tissue function.

Experiments reported in the present paper suggest that brown adipose tissue function is enhanced by corticotropin.

Experimental

Animals

The Zucker *fa/fa* rats and lean littermates *Fa/?* used in these experiments were bred from heterozygote parents (*Fa/fa*) in the University animal facility. All pups were weaned at 3 weeks of age and provided with rat chow (Christopher Hill, Poole, Dorset, U.K.) *ad libitum*. Rats used in these experiments were 4–5-week-old males.

Bilateral adrenalectomies and sham operations were performed as previously described 1 week before killing (Holt & York, 1982). Adrenalectomized rats were maintained on 0.9% (w/v) NaCl drinking solution and corticosterone (1 mg/day subcutaneously) as previously described (Holt *et al.*, 1983). Corticotropin [Synachthen-Depot; corticotropin-(1–24)-peptide absorbed to a zinc phosphate complex; Ciba Laboratories, Horsham, Sussex, U.K.] was given intramuscularly at the doses shown. Control animals received an equal volume of 0.9% (w/v) NaCl.

Methods

Interscapular brown adipose tissue mitochondria were prepared and specific binding of GDP was measured by incubating mitochondria with 10 μM -[^3H]GDP in the presence or absence of 100 μM -GDP as previously described (Holt *et al.*, 1983). The activity of succinate-cytochrome *c* oxidoreductase was used as a mitochondrial marker enzyme (Holt *et al.*, 1983). Serum corticosterone was measured by a radioimmunoassay procedure.

Each serum sample was assayed in duplicate based upon the method described by Fahmy *et al.* (1975).

Data were analysed for statistical significance by Student's *t* test.

Results

The specific binding of [^3H]GDP to isolated brown fat mitochondria has been used extensively to assess the activity of the thermogenic proton conductance pathway unique to these mitochondria (Rothwell *et al.*, 1982). The acute responses of brown adipose tissue mitochondrial GDP binding of 4-week-old lean and obese *fa/fa* rats to varying doses of corticotropin are shown in Table 1. In lean rats, a small increase in GDP binding (25%) was only observed at the highest treatment dose (100 μg). In contrast, the impaired GDP binding observed in obese rats was increased more than 2-fold after a single injection of 50 μg of corticotropin, to reach levels similar to those observed in control lean rats. A smaller (39%) increase in GDP binding was observed in obese rats given the maximum dose (100 μg) of corticotropin. Scatchard analysis of GDP binding at various concentrations of GDP showed that corticotropin had no effect on the affinity of GDP for its binding site (K_D 1.67 and 1.62 μM for obese control and obese + corticotropin respectively) but did increase maximum binding (B_{max} 162 and 332 pmol/mg of protein for obese control and obese + corticotropin respectively). Corticotropin treatment was not associated with any acute changes in the mitochondrial content of brown fat in either lean or obese rats, as indicated by the stability of tissue succinate-cytochrome *c* oxidoreductase activity.

Serum corticosterone concentrations were similar in lean and obese rats (Table 1). The time course of the response of serum corticosterone to corticotropin appeared to differ in the two groups of rats, the initial increase occurring earlier (12 h) in the obese than in the lean (24 h) rats. Such differences may reflect varying rates of absorption of the intramuscularly injected corticotropin.

Since increasing corticosterone concentrations are normally associated with a reduction in brown adipose tissue thermogenic function (Galpin *et al.*, 1983), the results suggested that brown adipose tissue GDP binding was increased by corticotropin and that the obese rat was particularly sensitive to such stimulation. To study the effect of corticotropin further, the time course of the response to corticotropin (50 $\mu\text{g}/\text{day}$) was investigated (Table 2). No significant effects on GDP binding were observed 12 h after corticotropin. The peak level of GDP binding in obese rats was observed after 24 h when the GDP binding level reached that observed in lean rats. Subsequently, as corticotropin administration continued, a significant rise in serum corticosterone levels was evident in both groups of rats and this was accompanied by a decrease in mitochondrial GDP binding to or towards the low level observed in untreated obese rats in both lean and obese rats. The response time to corticotropin was not shortened by using a corticotropin preparation that was absorbed more rapidly than Synachthen-Depot (I. Al-Baker, unpublished work). Corticotropin treatment was associated with a rapid (within 24 h) and sustained decrease in food intake of obese rats from 20.7 ± 2.6 to 15 ± 1.6 g/day (means $\pm$ S.E.M. for four rats in each group). Food intake of lean rats was unaffected by corticotropin (control 14.6 ± 0.3 ; corticotropin-treated 14.5 ± 0.8 g/day; means $\pm$ S.E.M. for four rats in each group).

In order to confirm the hypothesis that corticosterone and corticotropin have opposing effects on brown adipose tissue thermogenic function it was necessary to demonstrate clearly a corticotropin stimulation of GDP binding in lean rats. Lean and obese rats were adrenalectomized and maintained on either NaCl drinking solution (0.9% w/v) alone or together with corticosterone replacement (1 mg/day for 7 days). This latter treatment gave serum corticosterone concentrations of 2.5 ± 0.4 and 1.8 ± 0.3 $\mu\text{g}/\text{dl}$ (mean $\pm$ S.E.M.), 24 h after the last corticosterone injection, for eight lean and eight obese rats respectively. The acute response to

Table 1. *Brown adipose tissue GDP binding and serum corticosterone in lean and obese (fa/fa) rats treated with corticotropin*
Male rats (4–5-week-old) received a single dose of corticotropin or saline intramuscularly 24 h before killing. Results are means $\pm$ S.E.M. for the numbers of rats shown in parentheses. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with control (no corticotropin) group.

Parameter and unit	Rats	Corticotropin ($\mu\text{g}/\text{day}$)	None (control)	25	50	100
Serum corticosterone ($\mu\text{g}/\text{dl}$)	Lean		23 ± 2 (4)	—	39 ± 7 (8)	43 ± 7 (12)
	Obese		22 ± 2 (4)	—	27 ± 4 (8)	33 ± 1 (8)
GDP binding (pmol/mg of mitochondrial protein)	Lean		262 ± 14 (4)	285 ± 14 (8)	260 ± 22 (12)	328 ± 5 (12)
	Obese		114 ± 4 (4)	112 ± 10 (8)	240 ± 13 (8)	159 ± 123 (8)
Succinate-cytochrome <i>c</i> oxidoreductase ($\mu\text{mol}/$ min per tissue)	Lean		2.31 ± 0.15 (4)	—	2.63 ± 0.23 (12)	2.48 ± 0.18 (12)
	Obese		1.45 ± 0.10 (4)	—	1.65 ± 0.21 (8)	1.62 ± 0.17 (8)

Table 2. Time course of the response of brown adipose tissue GDP binding and serum corticosterone to corticotropin in lean and obese (*fa/fa*) rats

Corticotropin (50 µg of Synachthen-Depot/day) was injected intramuscularly. GDP binding of mitochondria prepared from interscapular brown adipose tissue was measured as described in the Experimental section. Rats were 4 weeks old at the beginning of the study. Values represent means ± S.E.M. for four rats in each group. **P* < 0.05, ****P* < 0.001 compared with lean group; †*P* < 0.05, ††*P* < 0.01, †††*P* < 0.001 compared with zero-time group.

Time (h)	GDP bound (pmol/mg of mitochondrial protein)		Serum corticosterone (µg/dl)	
	Lean	Obese	Lean	Obese
0	274 ± 22	86 ± 8***	23 ± 2	22 ± 2
12	271 ± 8	111 ± 12***	23 ± 2	39 ± 4††
24	260 ± 26	240 ± 15†††	39 ± 7	27 ± 4
72	144 ± 9†	191 ± 26††	46 ± 9	51 ± 2*†††
120	139 ± 15††	179 ± 5*††	81 ± 3†††	110 ± 7*†††
168	90 ± 7†††	131 ± 8*†	—	—

Table 3. Effect of corticotropin on brown adipose tissue GDP binding in adrenalectomized lean and obese rats maintained on corticosterone

Lean and obese (*fa/fa*) rats were adrenalectomized at 4 weeks of age and maintained on corticosterone (1 mg/day, subcutaneously). After 6 days, rats were given either 50 µg of corticotropin or saline vehicle intramuscularly before killing 24 h later. Brown adipose tissue mitochondrial GDP binding was assayed as described in the Experimental section. Values represent means ± S.E.M. for five rats in each group. ***P* < 0.01, ****P* < 0.001 compared with lean group.

	GDP bound (pmol/mg of mitochondrial protein)		
	Lean	Obese	
Adrenalectomized control	240 ± 20	238 ± 24	} <i>P</i> < 0.05 } <i>P</i> < 0.001 } <i>P</i> < 0.05
Adrenalectomized + corticosterone	246 ± 10	161 ± 31**	
Adrenalectomized + corticosterone + corticotropin	317 ± 15	232 ± 15***	

corticotropin (50 µg) of GDP binding in adrenalectomized lean and obese rats maintained on corticosterone is shown in Table 3. In confirmation of a previous report (Holt *et al.*, 1983) corticosterone replacement had no effect on GDP binding in adrenalectomized lean rats but suppressed GDP binding in adrenalectomized obese rats. However, in both groups, a corticotropin stimulation of GDP binding was observed when endogenous corticosterone secretion was absent. Although the increase in GDP binding was identical in both groups (71 pmol/mg of protein) this represented a larger percentage increase in the obese group (44% versus 29%).

Discussion

The obesity of the *fa/fa* rat is thought to result from the failure to increase brown adipose tissue thermogenesis in response to dietary signals (Holt *et al.*, 1983; Rothwell *et al.*, 1981; Triandafillou & Himms-Hagen, 1983). This defect, reflected in a reduction in brown adipose tissue mitochondrial GDP binding, is present shortly after birth (Bazin

et al., 1984; York *et al.*, 1984b) and shows a close relationship to gene dosage (York *et al.*, 1984b). Since the defective brown fat thermogenesis of obese *fa/fa* rats is rectified after adrenalectomy, a procedure which prevents the development of obesity and normalizes energetic efficiency (Yukimura *et al.*, 1978; Marchington *et al.*, 1983), the present studies were performed to investigate the possibility that corticotropin might regulate brown fat thermogenesis. Previous work had shown that corticosterone, but not aldosterone, replacement to adrenalectomized *fa/fa* rats could restore the defective brown adipose tissue GDP binding (Holt *et al.*, 1983; York *et al.*, 1984a) and that corticosterone would inhibit diet-related brown adipose tissue function in mice (Galpin *et al.*, 1983).

The experimental data presented in the present paper would suggest that corticotropin and corticosterone have opposing stimulatory and inhibitory effects respectively on brown fat mitochondrial GDP binding. In the lean rat, this corticotropin stimulation could only be demonstrated when the opposing corticosterone-inhibitory effect was prevented by adrenalectomy. In the

obese rat the corticotropin stimulation of GDP binding was observed after 24h when no parallel increase in corticosterone was present and despite a reduction in food intake. However, the corticotropin stimulation of GDP binding in obese rats was attenuated at high doses of corticotropin and after prolonged treatment, when serum corticosterone levels were also raised. Previous reports have shown that both acute infusions of and chronic treatment with corticotropin may increase metabolic rate in rats (Jansky, 1973; Laurey & Portet, 1977). It has been difficult to explain the glucocorticoid-dependence of the obesity of Zucker *fa/fa* rats since serum corticosterone concentrations have been shown to be normal in the young rat during the period of excessive fat deposition, an observation confirmed in the present experiments (Shargill *et al.*, 1983; Yukimura *et al.*, 1978). However, Yukimura *et al.* (1978) have reported that plasma corticotropin concentrations of *fa/fa* rats are normal, consistent with their normal corticosterone concentrations. Thus, although it would now appear that the restoration of brown fat thermogenic capacity in obese rats following adrenalectomy is mediated by both the increase in corticotropin and the decrease in corticosterone, the cause of the defective brown fat function in the intact obese rat which has normal circulating levels of both these hormones remains unclear.

Diet-induced changes in brown adipose tissue thermogenesis are mediated through the sympathetic nervous system (Landsberg & Young, 1982). Recent investigations have shown that sympathetic stimulation of brown adipose tissue is impaired in obese rats but restored to normal after adrenalectomy (Levin *et al.*, 1982; D. Marchington & D. A. York, unpublished work). Since brown adipose tissue sympathetic activity is also reduced in corticosterone-treated lean rats it seems likely that corticosterone and/or corticotropin may be important in regulating the sympathetic response to dietary signals. However, since corticosterone

secretion, and presumably also corticotropin, has been shown to increase after feeding (Brindley *et al.*, 1979) it is possible that these two hormones have an important function in regulating the thermogenic response of brown adipose tissue after feeding.

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