

The effect of adrenalectomy on GDP binding to brown-adipose-tissue mitochondria of obese rats

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GDP binding to brown-adipose-tissue mitochondria of young obese Zucker rats (*fa/fa*) was significantly lower than in lean control rats, as a result of a decrease in the number of binding sites. Adrenalectomy of *fa/fa* rats restored GDP binding to control values. Corticosterone replacement suppressed GDP binding in adrenalectomized obese rats.

It has become evident from a wide range of studies that the regulation of brown-adipose-tissue thermogenesis is an important mechanism for controlling energy balance and preventing obesity. The thermogenic capacity of brown adipose tissue is thought to reside in the unique ability of brown-adipose-tissue mitochondria to translocate protons across the mitochondrial inner membrane without coupling to ATP synthesis, the so-called proton-conductance pathway (Nicholls, 1979). This pathway, which is inhibited by purine nucleotides (GDP and ADP) binding to a 32 000-mol.wt. protein (Heaton *et al.*, 1978; Nicholls, 1979, can be monitored by the specific binding of [³H]GDP to brown-adipose-tissue mitochondria.

The genetic obesities of the obese (*ob/ob*) mouse, the diabetes (*db/db*) mouse and the fatty (*fa/fa*) rat are all characterized by a fall in rectal temperature and an impairment of the thermogenic response to cold exposure (Bray & York, 1979; Levin *et al.*, 1980; Trayhurn & James, 1978; Trayhurn, 1979; York *et al.*, 1972). GDP-binding studies have been used to show that the thermogenic capacity of brown-adipose-tissue mitochondria is decreased in both *ob/ob* and *db/db* mice and that this defect may be one of the earliest detectable changes associated with the mutant genes (Himms-Hagen & Desautels, 1978; Goodbody & Trayhurn, 1982). An increase in the thermogenic capacity of the brown-adipose-tissue mitochondria is observed in the overfed 'cafeteria' rat, and this increase in brown-adipose-tissue thermogenesis is associated in young rats with an ability to maintain near-normal body weight and composition despite excessive dietary energy intake (Rothwell & Stock, 1980; Stock & Rothwell, 1982). This adaptation to overfeeding is similar to the responses observed on cold exposure, both being

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controlled by the autonomic nervous system and thyroid hormones (Rothwell & Stock, 1981).

The precise defect causing the thermogenic incapacity of brown-adipose-tissue mitochondria in obese (*ob/ob*) and diabetic (*db/db*) mice is unclear. The acute increase in GDP binding on cold exposure is absent in *ob/ob* mice, although GDP binding is increased after exposure to moderate cold over a 2-week period (Hogan & Himms-Hagen, 1980). Overfeeding of both obese (*ob/ob*) mice (Trayhurn *et al.*, 1982) and obese (*fa/fa*) rats (Rothwell *et al.*, 1982) does not cause the expected increase in GDP binding and metabolic rate respectively. A decrease in sympathetic activity and an increase in parasympathetic activity has been proposed as the basis of this impairment in obese rats (Rothwell *et al.*, 1982). However, it is known that adrenalectomy prevents the obesity of the Zucker obese rat (Yukimura & Bray, 1978; Yukimura *et al.*, 1978; Bray, 1982). It abolishes the hyperphagia and decreases serum insulin and hepatic lipogenesis to values close to those in lean animals (York & Godbole, 1979). However, obese rats still deposit excess fat stores when their food intake is paired to that of lean control rats (Bray *et al.*, 1973; Radcliffe & Webster, 1976). This suggests that adrenalectomy must enhance thermogenesis as well as decrease food intake in order to prevent obesity. Thus we report the effects of adrenalectomy on brown-adipose-tissue [³H]GDP binding in the *fa/fa* obese rat. The results suggest that the thermogenic capacity of brown-adipose-tissue is suppressed by adrenal steroids in the obese rat.

Materials and methods

Lean (+/+ and +/-) and obese (*fa/fa*) male rats (5 weeks old), bred from heterozygote (*fa/+*)

parents in the University animal facility, were housed at 24°C with a 12h-light/12h-dark cycle. All animals were fed on chow diet (Christopher Hill, Poole, Dorset, U.K.) *ad libitum* from weaning at 3 weeks of age.

Bilateral adrenalectomies and sham adrenalectomies were performed from the dorsal approach under Valium (0.25 mg/rat intraperitoneally) (Roche Products, Welwyn Garden City, Herts., U.K.)/fentanyl-fluanisone (1 mg/rat intramuscularly) (Crown Chemical Co., Lamberhurst, Kent, U.K.) anaesthesia. Adrenalectomized rats were maintained on 0.9% (w/v) NaCl in drinking water. Corticosterone (1 mg/day) was injected subcutaneously in an ethanol/dimethylformamide/0.9% (w/v) NaCl (2:1:7, by vol.) vehicle. Food intake was measured by placing the rats in individual metabolism cages on days 4 and 5 after surgery.

Mitochondria were prepared from the interscapular brown adipose tissue by the method of Slinde *et al.* (1975) after homogenization in Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] (1 mM)/EDTA (0.2 mM)/sucrose (0.25 M) buffer, pH 7.2 at 0°C. All mitochondrial preparations were washed with 2% (w/v) bovine serum albumin (fatty acid-free) solution in Hepes/EDTA/sucrose buffer.

Succinate-cytochrome *c* oxidoreductase (Complex II-III) activity, a specific mitochondrial-inner-membrane marker enzyme, was assayed on both the brown-adipose-tissue homogenate and the mitochondrial pellet by the method of Tisdale (1967).

Specific [³H]GDP binding was assayed by the method of Nicholls (1976) at a concentration of 10 μM-[³H]GDP (1.25 μCi) (The Radiochemical Centre, Amersham, Bucks., U.K.; sp. radioactivity 10 Ci/mmol) and 100 μM-potassium atractyloside in

the presence and absence of 100 μM-GDP. All incubation tubes contained 0.2 μCi of [U-¹⁴C]sucrose (sp. radioactivity 584 Ci/mol; The Radiochemical Centre) for estimation of extramitochondrial water. After incubation for 8 min at 25°C, the mitochondrial pellet was sedimented by centrifugation in a Beckman Microfuge at full speed for 2 min. Pellets were resuspended in NCS tissue solubilizer (The Radiochemical Centre), added to toluene/butyl-PBD [5-(biphenyl-4-yl)-2-(4-*t*-butylphenyl)-1-oxa-3,4-diazole] scintillant (G. & G. Chemicals, Ascot, Berks., U.K.) and counted for radioactivity in a Philips scintillation counter.

Scatchard analysis of [³H]GDP binding was performed as described above, with [³H]GDP concentrations ranging from 0.5 to 20 μM. [³H]-GDP binding was rapid and reached maximal values within 4 min in all groups.

Results and discussion

The weight of the interscapular brown-adipose-tissue depot is increased in 5-week-old obese Zucker rats (Table 1). This probably reflects an increase in their fat content, since the total protein content of the brown-adipose-tissue depot was slightly decreased in the obese rats. These results confirm the observations of Lavau *et al.* (1982). Similarly, the lower total activity of succinate-cytochrome *c* oxidoreductase activity (Table 1), a mitochondrial marker enzyme, in the brown adipose tissue of obese rats would indicate that the mitochondrial content of brown adipose tissue was also slightly decreased, although again this difference did not reach statistical significance. This interpretation is supported by the observation that the activity of succinate-cytochrome *c* oxidoreductase in the isolated mito-

Table 1. *Effect of adrenalectomy on body weight, food intake and brown adipose tissue of lean and obese (fa/fa) rats* Rats (5 weeks old) were adrenalectomized or sham-operated 7 days before they were killed, and maintained until then as described in the Materials and methods section at a housing temperature of 26°C. Brown-adipose-tissue mitochondria were incubated with 10 μM-[³H]GDP for 8 min at 25°C as described. Specific binding was assayed by the displacement of [³H]GDP with 100 μM-GDP. Values represent means ± s.e.m. for *n* animals. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 compared with lean rats in the same treatment group; †*P* < 0.05, ††*P* < 0.01, †††*P* < 0.001, compared with obese sham-operated rats.

| | Sham-operated | | | Adrenalectomized | | |
|--|---------------|--------------|------------------------|------------------|--------------|------------------------|
| | <i>n</i> | Lean | Obese (<i>fa/fa</i>) | <i>n</i> | Lean | Obese (<i>fa/fa</i>) |
| Body wt. (g) | 12 | 115.0 ± 7.0 | 139.0 ± 11.0 | 12 | 112.0 ± 10.0 | 121.0 ± 7.0 |
| Food intake (g/day) | 6 | 15.0 ± 1.8 | 21.1 ± 2.0* | 6 | 14.8 ± 2.1 | 14.5 ± 1.5† |
| Brown adipose tissue | | | | | | |
| Wet wt. (g) | 12 | 0.28 ± 0.02 | 0.50 ± 0.03*** | 12 | 0.28 ± 0.02 | 0.42 ± 0.02*** |
| Protein (mg) | 12 | 22.1 ± 1.8 | 17.4 ± 1.6 | 12 | 19.2 ± 1.6 | 24.2 ± 1.1***†† |
| Succinate-cytochrome <i>c</i> reductase (μmol/min) | 12 | 1.56 ± 0.32 | 0.97 ± 0.20 | 12 | 1.14 ± 0.15 | 1.16 ± 0.18 |
| [³ H]GDP binding (pmol/mg of protein) | 23 | 291.9 ± 14.7 | 149.0 ± 10.6*** | 13 | 279.0 ± 27.6 | 245.7 ± 20.2††† |

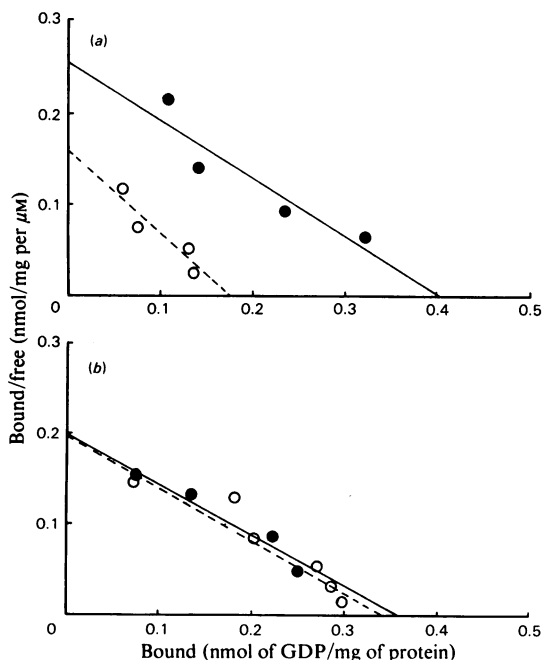


Fig. 1. Scatchard analysis of [^3H]GDP binding to brown-adipose-tissue mitochondria of lean (●) and obese (○) rats before (a) and after (b) adrenalectomy

Rats were killed and brown-adipose-tissue mitochondria were prepared 7 days after adrenalectomy. Other details of the experimental procedure are given in the Materials and methods section.

chondria was similar in lean and obese rats (results not shown).

Adrenalectomy had no significant effects on the weight of brown adipose tissue of lean rats or on its protein content or its mitochondrial content (as indicated by succinate-cytochrome *c* oxidoreductase activity) (Table 1). However, 7 days after adrenalectomy, the weight of brown adipose tissue of obese rats showed a small but significant fall towards values seen in lean rats, despite a significant increase in protein content. These changes presumably reflect a loss of tissue lipid stores, since the rate of lipogenesis is increased in brown adipose tissue of obese rats (Lavau *et al.*, 1982) and since adrenalectomy has been shown to result in a decrease in the rate of lipogenesis in both the liver and adipose tissue of obese rats (York & Godbole, 1979). However, the small increase in brown-adipose-tissue mitochondrial protein of obese rats after adrenalectomy, as indicated by the increase in activity of succinate-cytochrome *c* oxidoreductase, did not reach statistical significance.

The specific binding of [^3H]GDP to brown-adipose-tissue mitochondria was decreased by 50%

in obese rats (Table 1). This difference did not result from differing purity of mitochondrial preparations, since the specific activity of succinate-cytochrome *c* reductase was similar in all mitochondrial preparations (0.11 and 0.12 $\mu\text{mol}/\text{min}$ per mg of protein for lean and obese animals respectively). Thus the obese 'fatty' rat is similar to the *ob/ob* and *db/db* mice (Himms-Hagen & Desautels, 1978; Goodbody & Trayhurn, 1981) in having an apparent decrease in the capacity of the proton-conductance pathway in the brown-adipose-tissue mitochondria. Similarly, all these obese mutants are characterized by a decreased rectal temperature and an impairment in their thermogenic responses to cold exposure (Bray & York, 1979).

Scatchard plots (Fig. 1) of GDP binding to brown-adipose-tissue mitochondria from lean and obese rats were linear ($r = 0.93$), suggesting that only one type of binding site was present. The apparent dissociation constants (1.1 μM for obese and 1.6 μM for lean animals) were close to those reported for *db/db* mice (Goodbody & Trayhurn, 1981) and for rats (Sundin & Cannon, 1980) and suggest that there is not a major change in affinity for GDP binding in obese rats. However, the maximum binding capacity was decreased from 405 pmol/mg in lean rats to 175 pmol/mg in obese rats. These results suggest that there is a large fall in the number of GDP-binding sites in the brown adipose tissue of obese rats. This could result either from a decrease in the amount of the 32000-mol.wt. protein in the mitochondria or to masking of those binding sites.

Adrenalectomy had no significant effect on mitochondrial GDP binding in lean rats (Table 1). However, after adrenalectomy of obese rats there was a large increase in mitochondrial GDP binding, to values approaching those observed in lean control and adrenalectomized rats.

Scatchard analysis of GDP binding again revealed linear plots ($r = 0.97$ for lean and $r = 0.98$ for obese animals) after adrenalectomy, with apparent dissociation constants (K_D) slightly higher than those observed in their respective control rats (1.8 μM for lean, 1.7 μM for obese). The maximum binding capacity of brown-adipose-tissue mitochondria was slightly decreased by adrenalectomy of lean rats (405 and 360 pmol/mg for control and adrenalectomized rats respectively), but was increased from 175 to 345 pmol/mg in obese rats after adrenalectomy. These results suggest that the activity of the proton-conductance pathway in brown adipose tissue of obese rats is decreased, but may be rectified by adrenalectomy. The normalization of weight gain after adrenalectomy of the *fa/fa* rat may thus result from both the decrease in food intake (Yukimura *et al.*, 1978; York & Godbole, 1979) and from an increase in brown-adipose-tissue thermogenesis.

Corticosterone treatment of adrenalectomized obese rats restores their hyperphagia (Yukimura *et al.*, 1978). Similarly, corticosterone treatment (1 mg/day for 5 days) suppressed the brown-adipose-tissue mitochondrial GDP binding in adrenalectomized obese rats (250 ± 12 pmol/mg for four adrenalectomized obese rats and 99 ± 10 pmol/mg for four corticosterone-treated adrenalectomized obese rats), but had no effect in lean rats (270 ± 36 and 257 ± 28 pmol/mg for five control and five corticosterone-treated rats respectively). This apparent glucocorticoid suppression of GDP binding is surprising, since the thermogenic response to cold exposure is enhanced by glucocorticoids (Deavers & Musacchia, 1979). However, this effect may reflect primarily the permissive effects of glucocorticoids on catecholamine-induced mobilization of non-esterified fatty acids, on shivering and on piloerection. An alternative interpretation of the results would suggest that the increase in corticotropin secretion that would be expected after adrenalectomy might be the endocrine effector of the GDP-binding changes in obese rats.

Although one report has suggested that serum corticosterone concentration is increased in obese rats at certain times of the diurnal cycle (Martin *et al.*, 1978), other investigations have failed to confirm this and have also shown normal concentrations of serum corticotropin (Yukimura *et al.*, 1978; N. S. Shargill & D. A. York, unpublished work). Thus, although both the hyperphagia and depressed thermogenic capacity of brown-adipose-tissue mitochondria of obese rats require the presence of adrenal corticosteroids for their expression, the mechanism of this/these effect(s) remains obscure.

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