Biochem. J. (1983) **214**, 265–268
Printed in Great Britain

GDP binding to brown-adipose-tissue mitochondria of mice treated chronically with corticosterone

Katherine S. GALPIN,* Robert G. HENDERSON,† W. Philip T. JAMES*‡ and Paul TRAYHURN*§
*M.R.C. Dunn Nutrition Laboratory, Milton Road, Cambridge CB4 1XJ, U.K., and †Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, U.K.

(*Received 5 April 1983/Accepted 3 May 1983*)

Cytochrome oxidase activity and mitochondrial GDP binding were decreased in brown adipose tissue of mice treated chronically with corticosterone. These changes occurred both in corticosterone-treated mice fed *ad libitum* and in treated mice pair-fed to control animals. Although the dietary stimulation of brown-adipose-tissue thermogenesis was suppressed by corticosterone, the acute response to cold was not affected.

Corticosteroid administration to patients is frequently accompanied by a rapid gain in body weight and the development of obesity (Royal College of Physicians, 1983), and obesity is associated with the hypersecretion of corticosteroids in Cushing's syndrome. In experimental animals the administration of corticosteroids may also lead to weight gain, although there appear to be marked variations in response between strains (Hausberger & Hausberger, 1960). In a recent energy-balance study on mice, chronic treatment with corticosterone was found both to stimulate appetite and to increase metabolic efficiency (Galpin *et al.*, 1983). The increase in efficiency was demonstrated by pair-feeding the treated mice to the '*ad-libitum*' energy intake of control animals. In keeping with these observations, adrenalectomy has been shown to abolish hyperphagia and to inhibit the further development of obesity in several different obese mutants (see Bray & York, 1979; Bray, 1982).

Studies on genetically obese mice and on 'cafeteria'-fed rats have led to the view that thermogenesis in brown adipose tissue may be important in the regulation of energy balance and prevention of obesity, at least in small mammals (Himms-Hagen & Desautels, 1978; Rothwell & Stock, 1979; Goodbody & Trayhurn, 1981, 1982). The principal mechanism for thermogenesis in brown adipose tissue is through a proton conductance pathway across the mitochondrial inner membrane, the activity of which can be monitored by the binding of purine nucleotides such as GDP to an 'uncoupling' protein of mol.wt. 32000 (for review, see Nicholls, 1979). Although GDP binding to brown adipose tissue mitochondria is decreased in obese Zucker (*fa/fa*) rats, the binding is restored to normal after adrenalectomy, suggesting that glucocorticoids inhibit thermogenesis in brown adipose tissue in this mutant (Holt & York, 1982). However, adrenalectomy was reported to have no effect on mitochondrial GDP binding in lean rats (Holt & York, 1982).

We report here the results of a GDP binding study on brown-adipose-tissue mitochondria from mice that deposit excess body fat after the chronic administration of corticosterone. Results are presented both for treated mice fed *ad libitum* and for mice pair-fed to the normal intake of control animals.

Experimental

Animals

Male C57BL10ScSn mice, bred at the Dunn Nutrition Laboratory, were used at 2½ months of age. They were housed individually in wire-mesh cages in a room at 22 ± 1°C, with a 12h light/12h dark cycle (light period from 07:00h). The cages were suspended above absorbent paper to facilitate the collection of food spillage and faeces.

The mice were fed a synthetic diet containing 25% (w/w) protein and 10% (w/w) fat. Two groups of mice received 0.35mg of corticosterone acetate (Sigma London Chemical Co., Poole, Dorset, U.K.) per day, which was administered in the diet. The dose of corticosterone acetate given was calculated to be equivalent to approximately three times the replacement level for adrenalectomized rats. The control mice were fed *ad libitum*, as was one of the corticosteroid-treated groups. The other group of treated mice was pair-fed to the '*ad-libitum*' intake of control animals matched for similar body weights. Pair-feeding was achieved by giving each cortico-

‡ Present address: Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, Scotland, U.K.

§ To whom correspondence and reprint requests should be addressed.

steroid-treated mouse the previous day's intake (adjusted for spillage) of the control animal. The concentration of corticosterone acetate in the diet was adjusted daily so that both the '*ad-libitum*' and pair-fed groups received the same dose.

In a separate overfeeding experiment, mice were fed either a stock diet (Spillers–Spratts Rodent Breeding Diet 1; Spratts Patent Ltd., Barking, Essex, U.K.) or a stock diet plus chocolate; a supplement of chocolate is a simple way of inducing hyperphagia in mice (Younger & Trayhurn, 1983).

Methods

Mice were killed by cervical dislocation and interscapular brown adipose tissue was rapidly removed and dissected free of other tissues. Cytochrome oxidase activity was measured spectrophotometrically at 30°C by the method of Yonetani & Ray (1965). Protein was measured by the method of Schacterle & Pollack (1973). For the preparation of mitochondria, brown adipose tissue was taken from the interscapular, subscapular and dorsocervical sites from each animal and pooled. Mitochondria were then isolated as described by Cannon & Lindberg (1979). The binding of purine nucleotides (GDP) was measured by incubating the mitochondria with 10μM-[^{3}H]GDP at room temperature, in a medium at pH 7.1, as described previously (Goodbody & Trayhurn, 1981). [^{3}H]-GDP and [^{14}C]sucrose were obtained from Amersham International (Amersham, Bucks., U.K.). Radioactivity was measured in a Packard Tri-Carb 2650 liquid-scintillation counter, with corrections made for background counts and quench.

The energy content of food and faeces was determined with an Adiabatic Bomb calorimeter (Gallenkamp and Co., London, U.K.).

The statistical significance of differences between groups was assessed by Student's unpaired *t* test.

Results

The body weight of the corticosteroid-treated mice fed *ad libitum* increased by 25% in 3 weeks after the start of the treatment (Table 1). During this time there was no significant change in the weight of either the control mice or the pair-fed treated mice. Body energy stores are, however, markedly increased in the pair-fed animals (Galpin *et al.*, 1983). The digestible energy intake was 17% higher in the corticosteroid-treated mice fed *ad libitum* than in the control animals.

The amount of interscapular brown adipose tissue was substantially increased in both the pair-fed and '*ad-libitum*' fed groups of corticosteroid-treated mice (Table 1). The protein content was not altered, however, indicating that the increased amount of interscapular brown adipose tissue in the treated animals is not due to a true hypertrophy of the tissue. Cytochrome oxidase activity, used as a mitochondrial marker, was significantly lower in the pair-fed mice than in the controls, and it was further decreased in the corticosteroid mice fed *ad libitum*.

The binding to GDP to mitochondria is now the principal method by which the activity of the thermogenic proton conductance pathway in brown adipose tissue is assessed. The results in Table 1 show that GDP binding is significantly decreased in both groups of corticosteroid-treated mice, compared with the control mice. A Scatchard plot of GDP binding at different concentrations of GDP showed no difference in affinity between corticosteroid-treated and control mice (K. S. Galpin, unpublished work).

There was little difference in GDP binding between the pair-fed mice and the treated mice fed *ad libitum*, despite the greater energy intake of the latter group. This suggests that any dietary-induced thermogenesis invoked by overfeeding may be suppressed by corticosteroids in these animals.

Table 1. *Cytochrome oxidase activity and mitochondrial GDP binding of brown adipose tissue from corticosterone-treated mice*

The treated mice received 0.35 mg of corticosterone acetate/day for 21 days; for full experimental details see the text. The GDP-binding assay was performed on mitochondria isolated from brown adipose tissue pooled from the interscapular, subscapular and dorsocervical sites. The initial body weight of the mice was 31 g. Results are means ± S.E.M. for the numbers of observations shown in parentheses. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, compared with controls.

		Corticosterone-treated	
	Controls	Pair-fed to controls	Fed *ad libitum*
Body wt. of mice (g)	31.2 ± 0.7 (6)	31.0 ± 0.5 (7)	38.3 ± 1.0 (6)***
Interscapular brown adipose tissue wt. (mg)	159 ± 17 (6)	483 ± 30 (7)***	549 ± 37 (6)***
Protein content of interscapular brown adipose tissue (mg)	13.5 ± 0.6 (6)	14.2 ± 1.1 (7)	13.8 ± 1.1 (6)
Cytochrome oxidase activity of interscapular brown adipose tissue (μmol of cytochrome *c* oxidized per min)	16.9 ± 1.2 (6)	12.9 ± 1.1 (7)*	9.8 ± 1.2 (6)**
GDP bound (pmol/mg of mitochondrial protein)	353.7 ± 64.3 (5)	176.2 ± 6.2 (7)*	197.7 ± 19.9 (7)*

Table 2. *Cytochrome oxidase activity and mitochondrial GDP binding of brown adipose tissue from overfed mice*
The mice received either stock diet or stock diet plus chocolate for 21 days; for full experimental details see the text. The GDP-binding assay was performed on mitochondria isolated from brown adipose tissue pooled from the interscapular, subscapular and dorsocervical sites. Results are means ± S.E.M. for the numbers of observations shown in parentheses. *$P < 0.05$, compared with controls.

	Controls (stock diet)	Overfed (stock diet + chocolate)
Digestible energy intake (kJ)	1259 ± 26 (6)	1455 ± 38 (14)*
Interscapular brown adipose tissue wt. (mg)	103 ± 7 (6)	163 ± 14 (7)*
Cytochrome oxidase activity of interscapular brown adipose tissue (μmol of cytochrome *c* oxidized/min)	19.4 ± 1.3 (6)	24.0 ± 1.1 (7)*
GDP bound (pmol/mg of mitochondrial protein)	229.9 ± 49.2 (9)	395.8 ± 26.5 (9)*

Table 3. *GDP binding to brown-adipose-tissue mitochondria from corticosterone-treated mice after acute exposure to cold*
The treated mice received 0.35 mg of corticosterone acetate/day for 21 days, and both the control and treated animals were exposed to a temperature of 4°C for 1 h. For full experimental details see the text. Results are means ± S.E.M. for the numbers of observations shown in parentheses. *$P < 0.05$, compared with similarly treated warm group.

	GDP bound (pmol/mg of mitochondrial protein)	
	Controls	Corticosteroid-treated
Warm (21°C)	306.5 ± 39.0 (6)	193.4 ± 16.1 (5)
Cold-exposed (4°C)	407.1 ± 24.6 (7)*	337.5 ± 48.8 (5)*

However, in order to substantiate this conclusion it is necessary to demonstrate that brown adipose tissue thermogenesis is stimulated by overfeeding in the particular strain of mice used in the present study, since diet-induced thermogenesis is not always observed in hyperphagic animals (Hervey & Tobin, 1982). Feeding C57BL10ScSn mice on a supplement of chocolate led to a 15% increase in digestible energy intake over stock-fed mice (Table 2), and this level of hyperphagia is similar to that observed in the corticosteroid-treated animals. The total tissue weight and cytochrome oxidase activity of interscapular brown adipose tissue were significantly higher in the mice overfed with chocolate, and mitochondrial GDP binding was also increased (Table 2). Thus the strain of mice used here clearly shows a stimulation of the currently accepted index of brown-adipose-tissue thermogenesis in response to overfeeding. The GDP binding values obtained for the control animals in the chocolate-overfeeding study were lower than in the previous experiment, and although there was no clear explanation for this difference the decreased fat content of the stock diet compared with the synthetic diet could be relevant.

In order to determine whether corticosteroid-treated mice also have an impaired thermogenic response to cold, control and treated animals (fed *ad libitum*) were placed at 4°C for 1 h and mitochondrial GDP binding was then measured. The results shown in Table 3 indicate that treatment with corticosterone does not lead to a suppression of the acute response to cold; GDP binding increased on cold exposure in both the control and corticosteroid-treated mice, and the increase in binding was greatest in the treated animals.

Discussion

The present study was conducted on mice that deposit excess fat and develop obesity in response to the chronic administration of corticosterone; the effect of corticosterone in these mice is to increase both food intake and metabolic efficiency on a normal intake (Galpin *et al.*, 1983). The results reported here are consistent with a suppression of brown adipose tissue thermogenesis by corticosterone in both the pair-fed and '*ad-libitum*'-fed states. This suggests that the effect of corticosterone on metabolic efficiency and fat deposition on a normal energy intake includes a decrease in energy expenditure on brown-adipose-tissue thermogenesis. A similar explanation has been advanced for the high efficiency shown on a normal energy intake of genetically obese (*ob/ob* and *db/db*) mice (Himms-Hagen & Desautels, 1978; Hogan & Himms-Hagen, 1980; Thurlby & Trayhurn, 1980; Goodbody & Trayhurn, 1981, 1982).

The present results also indicate that the diet-induced thermogenesis stimulated by overfeeding (Rothwell & Stock, 1979) is inhibited by corticosterone, although no inhibition of the acute thermogenic response to cold occurred. These findings parallel observations on rats made obese through lesions of the ventromedial hypothalamus (Hogan *et al.*, 1982), indicating that overfeeding is a less

potent stimulus to brown-adipose-tissue thermogenesis than cold exposure. The obese (*ob/ob*) mouse differs from the corticosterone-treated mouse and from rats with hypothalamic lesions in that although all three obese models appear to have a decreased capacity for diet-induced thermogenesis in brown adipose tissue (Hogan *et al.*, 1982; Trayhurn *et al.*, 1982), only the *ob/ob* mutant shows impaired cold-induced thermogenesis (Himms-Hagen & Desautels, 1978).

The results obtained in the present study are consistent with the report that adrenalectomy restores the level of GDP binding to normal in the obese Zucker rat, a change that was reversed by the administration of corticosterone (Holt & York, 1982). Adrenalectomy, however, was found to have no effect on GDP binding in normal lean rats (Holt & York, 1982).

The mechanism through which an inhibition of brown adipose tissue thermogenesis is mediated by corticosterone in mice is not clear, but the possibilities include a decrease in corticotropin secretion or in sympathetic activity. However, a direct action of brown adipose tissue itself may be an important mechanism, since glucocorticoid receptors have been found in the tissue (Feldman, 1978). Although it appears surprising that corticosterone should inhibit thermogenesis in brown adipose tissue (Holt & York, 1982), in view of the increased secretion of glucocorticoids which occurs at least transitorily during cold exposure (Deavers & Musacchia, 1979), the present results indicate that the inhibition principally affects diet-induced thermogenesis. If the cold-induced stimulation of brown adipose tissue is mediated mainly through the sympathetic nervous system, then our results suggest that corticosterone has little effect on this mechanism and that it affects only the sympathetic output responsive to dietary stimuli. Alternatively, diet-induced thermogenesis may have an important component that does not involve sympathetic activation.

K. S. G. was supported by the Addenbrooke's Artificial Kidney Patients Association.

References

Bray, G. A. (1982) *Proc. Nutr. Soc.* **41**, 95–108
Bray, G. A. & York, D. A. (1979) *Physiol. Rev.* **59**, 719–809
Cannon, B. & Lindberg, O. (1979) *Methods Enzymol.* **55**, 65–78
Deavers, D. R. & Musacchia, X. J. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 2177–2181
Feldman, D. (1978) *Endocrinology* **103**, 2091–2097
Galpin, K. S., Henderson, R. G., James, W. P. T. & Trayhurn, P. (1983) *Proc. Nutr. Soc.* **42** in the press
Goodbody, A. E. & Trayhurn, P. (1981) *Biochem. J.* **194**, 1019–1022
Goodbody, A. E. & Trayhurn, P. (1982) *Biochim. Biophys. Acta* **680**, 119–126
Hausberger, F. X. & Hausberger, B. C. (1960) *Am. J. Clin. Nutr.* **8**, 671–679
Hervey, G. R. & Tobin, G. (1982) *Proc. Nutr. Soc.* **41**, 137–153
Himms-Hagen, J. & Desautels, M. (1978) *Biochem. Biophys. Res. Commun.* **83**, 628–634
Hogan, S. & Himms-Hagen, J. (1980) *Am. J. Physiol.* **239**, E301–E309
Hogan, S., Coscina, D. V. & Himms-Hagen, J. (1982) *Am. J. Physiol.* **243**, E338–E344
Holt, S. & York, D. A. (1982) *Biochem. J.* **208**, 819–822
Nicholls, D. G. (1979) *Biochim. Biophys. Acta* **549**, 1–29
Rothwell, N. J. & Stock, M. J. (1979) *Nature (London)* **281**, 31–35
Royal College of Physicians (1983) *J. R. College Physicians (London)* **17**, 5–65
Schacterle, G. R. & Pollack, R. L. (1973) *Anal. Biochem.* **51**, 654–655
Thurlby, P. L. & Trayhurn, P. (1980) *Pflügers Arch.* **385**, 193–201
Trayhurn, P., Jones, P. M., McGuckin, M. M. & Goodbody, A. E. (1982) *Nature (London)* **295**, 323–325
Yonetani, T. & Ray, G. S. (1965) *J. Biol. Chem.* **240**, 3392–3398
Younger, K. M. & Trayhurn, P. (1983) *Int. J. Obes.* **7**, 94–95