Induction of functional uncoupling protein in guinea pigs infused with noradrenaline

Studies with isolated brown adipocytes

Sonia A. CUNNINGHAM and David G. NICHOLLS

Department of Biochemistry, University of Dundee, DD1 4HN, Scotland, U.K.

Continuous infusion of noradrenaline over the interscapular brown fat of guinea pigs maintained at thermoneutrality (28–32 °C) induces changes similar to those after cold-adaptation. (1) Multilocular fat droplets appear within the brown adipocytes. (2) The number of mitochondria per adipocyte and the total number of adipocytes both increase. (3) Noradrenaline addition to isolated adipocytes causes near maximal uncontrolled respiration. (4) The cells become more sensitive to fatty acid-induced uncoupling. (5) The tissue-specific uncoupling protein per mg of mitochondrial protein is increased 5-fold. Specific α - and β -agonists were also chronically infused. (6) Separate infusion of phenylephrine or isoprenaline was not able to stimulate mitochondriogenesis or hyperplasia. (7) Adipocytes from these animals could not be uncoupled by acute noradrenaline. (8) Simultaneous chronic infusion of phenylephrine and isoprenaline reproduced the effects of chronic noradrenaline infusion.

INTRODUCTION

The brown-fat-specific 32 kDa uncoupling protein acts as a regulatable proton short circuit across the mitochondrial inner membrane and provides the dissipative mechanism which underlies the enormous capacity of brown fat for heat production (for reviews, see Nicholls & Locke, 1984; Nicholls *et al.*, 1986). The uncoupling protein is inducible, and closely parallels the thermogenic capacity of the tissue during development, cold-adaptation and dietary stimulation (for review see Trayhurn & Nicholls, 1986).

Cold-adaptation of the young adult guinea pig has been closely monitored at the cellular level (Locke *et al.*, 1982b; Rafael *et al.*, 1986; Cunningham *et al.*, 1986). Relative to thermoneutral controls, the brown fat of animals maintained at 4–7 °C shows a 2-fold, 4-fold and 28-fold increase in adipocyte number, mitochondrial content and uncoupling protein respectively (Rial & Nicholls, 1984; Rafael *et al.*, 1986). The respiratory response of the total brown adipocytes to acute noradrenaline increases 30-fold (Rafael *et al.*, 1986), and this correlates well with estimates *in vivo* for the tissue (Brück, 1970).

The sympathetic nervous system is thought to mediate the chronic adaptative changes in brown adipose tissue during cold-acclimation (for review see Ricquier *et al.*, 1985). However, early attempts to mimic chronic sympathetic stimulus by repetitive noradrenaline injection were largely unsuccessful (LeBlanc & Villemare, 1970; Desautels & Himms-Hagen, 1979; Mory *et al.*, 1980). The first demonstration of the induction of the uncoupling protein in thermoneutral animals (Ricquier *et al.*, 1983) was achieved in rats implanted with cloned pheochromocytoma cells which release catecholamines. Mory *et al.* (1984) implanted osmotic pumps into rats in order to deliver noradrenaline continuously; they

reported both hyperplasia and selective synthesis of the uncoupling protein.

The present results confirm for the guinea pig that chronic noradrenaline administration to thermoneutral animals can mimic the proliferative changes which occur in the tissue during cold-acclimation. Thus the amount of uncoupling protein per mg of mitochondrial protein, mitochondrial protein per adipocyte and total adipocyte numbers are all elevated. Most importantly, however, the cells from the noradrenaline-infused animals are thermogenically competent, showing a near-maximal uncontrolled respiration *in vitro* after acute noradrenaline administration. Secondly, the cells display a greatly increased sensitivity to uncoupling by fatty acid, the physiological cytoplasmic second messenger mediating the activation of the uncoupling protein (Locke *et al.*, 1982a; Rial *et al.*, 1983; Cunningham *et al.*, 1986). Therefore the uncoupling protein is not only synthesized and inserted into the mitochondrial inner membrane, but also becomes functional in the cells from the infused animal. Thus no essential metabolic or bioenergetic link is missing which would render the adipocytes non-thermogenic.

Since the β_1 -adrenoceptor is primarily responsible for the acute thermogenic response (Bukowiecki *et al.*, 1980), the role of chronic β -adrenoceptor stimulation of the adaptative response has been investigated (Heick *et al.*, 1973; Arch *et al.*, 1984; Young *et al.*, 1984; Ricquier *et al.*, 1986). However, although infusion of β -agonist can induce hyperplasia (Heick *et al.*, 1973; Mory *et al.*, 1980) the mitochondrial concentrations of uncoupling protein remain unchanged (Mory *et al.*, 1980; Young *et al.*, 1984). Our results are consistent with these findings, but suggest that, in contrast with the acute respiratory response, activation of both α - and β -adrenoceptors may be obligatory for the induction of thermogenically competent cells.

Abbreviation used: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazine.

EXPERIMENTAL

'Mini-osmotic' pumps were supplied by Alza (Scientific Marketing, London N.1, U.K.). Radiochemicals were obtained from Amersham International, Amersham, Bucks., U.K. Collagenase (Worthington type II) was supplied by Lorne Laboratories, Bury St. Edmunds, Suffolk, U.K. Lubrol 17A17 was obtained from ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, U.K. All other reagents were from Sigma Chemical Co. (Poole, Dorset, U.K.)

Animals

Pregnant guinea pigs (Dunkin Hartley) were maintained at 22 °C under a 12 h/12 h light/dark cycle with food (FD-1 diet) and water *ad lib*. At birth the mother and litter were transferred to a chamber maintained in the thermoneutral range for the young animals (28–32 °C) with a 16 h/8 h light/dark cycle. After weaning (14 days), animals of either sex were caged separately. At the commencement of the experiment the animals (250 ± 10 g) were 30 ± 4 days old.

Alzet mini-osmotic pumps (model 2002) were used to provide 11 days of continuous drug administration ($0.47 \mu\text{g/h}$). Experimental animals received ($\pm$)-noradrenaline (520 nmol/h); ($-$)-isoprenaline (31 nmol/h); L-phenylephrine delivered simultaneously from the same pump at 31 and 307 nmol/h respectively. To minimize oxidation of the amines, the filling solutions also contained 0.1 M -ascorbate and 0.02 M -4,5-dihydroxy-1,3-benzenedisulphonic acid (Mory *et al.*, 1984). Pumps were implanted subcutaneously immediately above the interscapular fat-pad. Thermoneutral controls were implanted with mini-osmotic pumps filled with anti-oxidants alone. After implantation, animals were replaced in the 28–32 °C chamber. Animals for cold-adaptation were removed to a cold-chamber and maintained at 4–7 °C with a 12 h/12 h light/dark cycle. Body weight was recorded daily. Animals were killed on the morning of day 11.

Preparation of mitochondria and brown adipocytes

Guinea pigs were killed by cervical dislocation and decapitation. Interscapular brown fat was dissected, and adipocytes were prepared by the method of Locke *et al.* (1982b) with subsequent modifications (Rafael *et al.*, 1986). Mitochondria were prepared as previously described (Cunningham *et al.*, 1986) and finally resuspended in 250 mM -sucrose, 10 mM -Tes (sodium salt), $64 \mu\text{M}$ -albumin (bovine, fatty acid-free), pH 7.2. Mitochondrial protein was measured in the presence of Triton X-100 by the Lowry procedure as modified by Wang & Smith (1975), with bovine serum albumin as standard. Allowance was made for albumin in the resuspension medium.

Adipocyte respiration

Adipocytes were suspended at approx. $100\,000/\text{ml}$ in $700 \mu\text{l}$ of cell incubation medium containing 110 mM -NaCl, 5.5 mM -KCl, 5 mM - NaH_2PO_4 , 1.5 mM - KH_2PO_4 , 5 mM - NaHCO_3 , 10 mM -Tes (sodium salt), 1.4 mM - MgSO_4 , 1.5 mM - CaCl_2 , 10 mM -pyruvate, 10 mM -D-glucose, 10 mM -fructose and $64 \mu\text{M}$ -albumin (essentially fatty acid-free), pH 7.4, 37 °C, in a Hansa-Tech oxygen-electrode chamber. With each preparation of cells the minimum concentration of FCCP required for

maximal release of respiration was initially determined. This concentration was then used at the end of each respiratory trace to obtain uncontrolled respiratory rates.

Mitochondrial GDP binding

Mitochondria (0.3 mg/ml) were incubated at 30 °C for 2 min in $250 \mu\text{l}$ of 250 mM -sucrose/ 10 mM -Tes/ $16 \mu\text{M}$ -albumin/ $1.3 \mu\text{M}$ -rotenone/ $0.25\text{--}6 \mu\text{M}$ - $[^3\text{H}]\text{GDP}$ ($1.0 \mu\text{Ci/ml}$)/ $[^{14}\text{C}]\text{sucrose}$ ($0.3 \mu\text{Ci/ml}$), pH 7.0. Scatchard analysis was performed as described previously (Rial & Nicholls, 1984).

Cytochrome c oxidase

Cytochrome c oxidase activity (EC 1.9.3.1) was measured polarographically at 37 °C as described by Rafael (1983). Tissue, cells and mitochondria were solubilized in 50 mM - KH_2PO_4 / 1 mM -EDTA, pH 7.2, with Lubrol 17A17 (w/w) at 0.5%, 0.5% and 0.1% respectively.

RESULTS AND DISCUSSION

Osmotic pumps were implanted, or animals transferred to 4 °C, when the body weight was 250 ± 10 g. Animals exposed to 4 °C lost 25 g of body weight during the first day, and subsequently lagged behind the thermoneutral control by 30–50 g (Fig. 1). Animals implanted with noradrenaline-containing pumps also lost 25 g in the first day, although the subsequent weight deficit was less marked. Control pumps (containing antioxidant alone) produced no weight loss.

The basic parameters for the tissue from control,

Fig. 1. Influence of cold and noradrenaline infusion on guinea-pig weight

The animals were weighed daily 4 days before and 10 days after the commencement of the experiment. On day 0 animals were implanted with mini-osmotic pumps containing noradrenaline and anti-oxidants (●) or anti-oxidants alone (▲) and maintained at thermoneutrality. In addition animals were removed to a cold chamber for cold-adaptation (■). All points are means $\pm$ S.E.M. for 12 animals.

Table 1. Basal parameters for interscapular brown adipose tissue control, noradrenaline-infused and cold-adapted guinea pigs

Values represent the mean $\pm$ S.E.M. of 12 control, 12 noradrenaline-infused and 12 cold-adapted animals. For conditions see the Experimental section. Total cells and mitochondria were calculated from the cytochrome *c* oxidase activity per 10^6 cells, per mg of mitochondrial protein, and for the total dissected tissue.

	Control	Noradrenaline-infused	Cold-adapted
Tissue wet. wt. (g)	3.33 ± 0.14	1.40 ± 0.12	1.43 ± 0.09
Cytochrome oxidase (per mg of mitochondria)	6.42 ± 0.77	6.90 ± 0.45	7.02 ± 1.01
Cytochrome oxidase (units/ 10^6 cells)	3.22 ± 0.57	4.10 ± 0.29	6.10 ± 0.81
Mitochondria (mg/ 10^6 cells)	0.50 ± 0.12	0.64 ± 0.10	0.87 ± 0.19
Cytochrome oxidase (total)	113.2 ± 9.2	185.0 ± 15.9	342.0 ± 38.7
$10^{-6} \times$ No. of cells (total)	34.8 ± 7.6	46.2 ± 6.6	53.9 ± 14.0
Mitochondria (mg) (total)	14.0 ± 1.2	24.2 ± 1.4	48.1 ± 5.4
Cytochrome oxidase (units/g)	35.0 ± 3.2	139.0 ± 11.2	227.6 ± 23.3
$10^{-6} \times$ No. of cells/g of tissue	10.4 ± 2.3	33.0 ± 8.0	37.7 ± 9.8

noradrenaline-infused and cold-adapted animals are shown in Table 1. The interscapular brown-fat pad from noradrenaline-infused and cold-adapted animals was more vascularized and much darker in appearance than was control tissue. Noradrenaline infusion causes a large tissue weight loss (Table 1), and a transition of more than 50% of the adipocytes from unilocular to possessing multilocular triacylglycerol stores, with an associated decrease in cell size. These changes parallel those after 11 days of cold-adaptation (Table 1) and indicate an extensive lipid depletion.

Since the cytochrome *c* oxidase activity per mg of mitochondrial protein does not change significantly (Table 1), the increased activity per 10^6 cells indicates a proliferation of the mitochondria per cell, amounting to 27% for the noradrenaline-infused and 89% for the cold-adapted animals. Total cell number can be estimated, avoiding problems of recovery, by comparing cytochrome *c* oxidase per 10^6 cells with the total tissue activity (Table 1). The 33% hyperplasia caused by the infusion compares with the 55% increase seen upon cold-adaptation.

Scatchard analysis of GDP binding to isolated mitochondria

Uncoupling protein in isolated mitochondria may be quantified by Scatchard analysis of GDP binding to the high-affinity purine-nucleotide-binding site (Nicholls, 1976). Fig. 2 shows that there is no significant difference in the binding affinity of the uncoupling protein between the three groups, the K_d for GDP being close to $3 \mu\text{M}$ in each case. However, the difference in binding capacity is striking: 73 ± 4 , 344 ± 49 and 660 ± 50 mol/mg for control, noradrenaline-infused and cold-adapted animals respectively.

Fig. 2 shows that noradrenaline infusion into themoneutral guinea pig causes synthesis of uncoupling protein, as has been previously reported for the rat (Mory *et al.*, 1984). However, it is necessary to show that the synthesized uncoupling protein is functional, and that the catecholamine-induced changes in the tissue are sufficient in themselves to confer thermogenic competence. For this, experiments with intact adipocytes are required.

Fig. 2. Scatchard plot of the binding of [^3H]GDP

Mitochondria from the brown adipose tissue of control (●), noradrenaline-infused (▲) and cold-adapted (■) guinea pigs were incubated at 30°C in a sucrose-based medium. GDP was varied from 0.25 to $6 \mu\text{M}$ (see the Experimental section). Each point represents the means $\pm$ S.E.M. for six to eight preparations.

Respiratory stimulation of brown adipocytes by acute administration of noradrenaline

Noradrenaline-stimulated lipolysis is equally high in brown adipocytes from control and cold-adapted guinea pigs (Cunningham *et al.*, 1986). The greatly increased thermogenic response of cells after cold-adaptation is only due in part to the increase in mitochondria. The major factor is the increase in uncoupling protein per mitochondrion, allowing the full uncontrolled respiratory capacity to be expressed (Locke *et al.*, 1982a,b; Rial & Nicholls, 1984; Rafael *et al.*, 1986).

Fig. 3 shows representative traces for noradrenaline stimulation of cells from control, noradrenaline-infused

Fig. 3. Sensitivity of brown-adipocyte respiration to acute noradrenaline stimulation

Cells from control (a), noradrenaline-infused (b) or cold-adapted (c) guinea pigs were incubated in an oxygen electrode at 37 °C in cell incubation medium (see the Experimental section). Subsequent additions were made of 10 μ M-(+)-noradrenaline (N) and 12 μ M-FCCP. Values beside traces are the respiration (nmol/min per 10⁶ cells). A representative experiment is shown.

and cold-adapted guinea pigs. The subsequent addition of FCCP establishes the degree of residual respiratory control. It is immediately apparent that chronic noradrenaline infusion into thermoneutral guinea pigs

causes a transition of the brown adipocytes from the non-thermogenic state (with little response to acute noradrenaline and retained respiratory control) to the thermogenic state (with almost complete release of respiratory control and a consequent large respiratory stimulation). Table 2 reports the collected data from a number of preparations.

Respiratory stimulation of brown adipocytes by exogenous palmitate and synthetic protonophores

Cytoplasmic unesterified fatty acids not only are the main respiratory substrates for the thermogenically active brown adipocytes, but also act as cytoplasmic second messengers, activating the uncoupling protein (Locke *et al.*, 1982a,b; Rial *et al.*, 1983; Cunningham *et al.*, 1986). Thus the ability of unesterified fatty acids to uncouple isolated mitochondria (Locke *et al.*, 1982a; Rial & Nicholls, 1984; Cunningham *et al.*, 1986) and isolated adipocytes (Locke *et al.*, 1982b; Cunningham *et al.*, 1986) correlates with the mitochondrial complement of uncoupling protein. Table 2 shows the collected data for a number of experiments for the respiratory stimulation of cells after addition of sufficient palmitate to generate an extracellular unbound concentration of 185 nM (Cunningham *et al.*, 1986). When evaluated per 10⁶ cells, palmitate addition elicits a 3.5-fold greater stimulation from the brown adipocytes of the noradrenaline-infused compared with the control guinea pigs. As with the other parameters, this is intermediate between the control and cold-adapted values.

It has been suggested (Nedergaard & Cannon, 1984) that the greater sensitivity of cold-adapted brown adipocytes to uncoupling by exogenous fatty acids could merely be a consequence of a non-specific increase in sensitivity to uncouplers, perhaps owing to an increase in total mitochondrial-inner-membrane area. To investigate this possibility, titrations were performed with the synthetic protonophore FCCP (Fig. 4). When expressed per cell (Fig. 4a), an increased respiratory sensitivity to FCCP could be detected for the cold-adapted and noradrenaline-infused cells. However, when expressed in

Table 2. Respiratory parameters for brown adipocytes isolated from control, noradrenaline-infused and cold-adapted guinea pigs

For conditions see the Experimental section. Total respiratory capacity for the excised interscapular brown fat-pad was estimated from the increase in adipocyte respiration after administration of 10 μ M-noradrenaline and the total cell number (from Table 1). Results are means ($\pm$ S.E.M.) for at least six preparations for each condition.

	Control	Noradrenaline-infused	Cold-adapted
Respiration (nmol of O/min per 10 ⁶ cells)			
Basal	33 $\pm$ 0.6	43 $\pm$ 0.3	90 $\pm$ 6.0
+ 10 μ M-noradrenaline	108 $\pm$ 19.8	564 $\pm$ 126	1003 $\pm$ 93.6
+ 10 μ M-noradrenaline	484 $\pm$ 48.0	622 $\pm$ 119	1003 $\pm$ 93.6
+ 12 μ M-FCCP			
+ 185 nM-unbound palmitate	51 $\pm$ 6.2	124 $\pm$ 23	286 $\pm$ 26.3
Ratios			
Noradrenaline/FCCP	0.21 $\pm$ 0.017	0.94 $\pm$ 0.03	1.00 $\pm$ 0.0
Noradrenaline/cytochrome oxidase	0.03 $\pm$ 0.011	0.14 $\pm$ 0.02	0.16 $\pm$ 0.02
Palmitate/cytochrome oxidase	0.007 $\pm$ 0.002	0.02 $\pm$ 0.004	0.04 $\pm$ 0.005
Total respiratory capacity (nmol of O/min)	4.35 $\pm$ 1.4	27.9 $\pm$ 3.5	60.5 $\pm$ 8.1

Fig. 4. Sensitivity of coupled pyruvate respiration to FCCP

Brown adipocytes from (●) control, (▲) noradrenaline-infused and (■) cold-adapted animals were incubated at 37°C in cell incubation buffer (see the Experimental section) with the addition of 10 mM-pyruvate. Subsequently, the indicated concentrations of FCCP were added. (a) Data expressed per 10^6 cells; (b) data expressed per mg of intracellular mitochondrial protein. FCCP was titrated into the incubation in $2\ \mu\text{M}$ steps. For further details see the Experimental section. Each point represents the mean $\pm$ S.E.M. for at least six preparations.

relation to respiratory-chain activity (Fig. 4b), these differences disappeared. Thus there is a clear distinction between FCCP uncoupling, which remains constant per mg of mitochondrial protein (Fig. 4b), and palmitate uncoupling (Table 2), which increases by 200% and 400% respectively relative to the mitochondria of the noradrenaline-infused and cold-adapted adipocytes, and is in addition dependent on the presence of the uncoupling protein (Rial & Nicholls, 1984).

Calculated thermogenic capacity of the interscapular brown fat-pad *in vivo*

Hyperplasia, mitochondriogenesis and uncoupling protein synthesis can each increase the thermogenic capacity of brown fat. From the total cytochrome *c* oxidase activity of the dissected tissue (Table 1) and the relationship between cytochrome *c* oxidase activity and noradrenaline-stimulated respiration of the isolated adipocytes (Table 2), the total thermogenic capacity *in vivo* can be estimated. The potential for thermogenesis of the interscapular brown-fat pad in control animals is increased 6-fold by chronic noradrenaline infusion, to a value some 50% of that achieved after a similar period of cold-adaptation.

Adrenoceptor specificity for the chronic induction of thermogenically competent brown fat

α - and β -adrenoceptors have been characterized in isolated brown adipocytes from rat (Garcia-Sainz *et al.*, 1980; Bukowiecki *et al.*, 1980; Raasmaja *et al.*, 1985; Dominguez *et al.*, 1986) and fetal lamb (Fain *et al.*, 1984). Although the β -agonist isoprenaline can stimulate respiration in isolated hamster brown adipocytes, a minor α -adrenergic component can be revealed by the use of phenylephrine and can be blocked by the antagonist prazosin (Mohell *et al.*, 1983; Schimmel *et al.*, 1983).

Isoprenaline stimulation of guinea pig brown adipocyte respiration has a K_a of 10 nM, compared with 100 nM for noradrenaline (J. Rafael, unpublished work). In contrast with noradrenaline, isoprenaline is neither metabolized by monoamine oxidase nor taken up into adrenergic terminals. In view of these factors, and because higher rates proved toxic, isoprenaline was infused at 12% of the rate for noradrenaline. This is comparable with previous studies (Heick *et al.*, 1973; Mory *et al.*, 1980). Phenylephrine was infused at a similar rate to noradrenaline.

Table 3 shows the effect of separate and simultaneous infusion of isoprenaline and phenylephrine. In each case, although there is a decrease in the wet weight of the interscapular brown adipose tissue relative to the control, this is less marked than for the cold-adapted and noradrenaline-infused animals (Table 1). Phenylephrine and isoprenaline are, however, totally unable to induce cytochrome oxidase when infused separately, the activity being close to that obtained from the controls when expressed per g of tissue, and slightly lower when expressed per total interscapular depot. In contrast, isoprenaline and phenylephrine administered together increase the total cytochrome *c* oxidase activity of the pad by 270% (Table 3). Similarly, only those cells from animals infused with both agonists showed an enhanced uncoupling after acute noradrenaline treatment (Table 3). Evidently the combined action of both agonists is required to mimic cold-adaptation.

Conclusions

The present results show that chronic noradrenaline infusion into the interscapular brown fat of thermoneutral guinea pigs induces cell proliferation, mitochondriogenesis and uncoupling of protein synthesis, and allows functionally thermogenic cells to be isolated. Under the particular conditions of our noradrenaline-infusion study, the tissue weight loss caused by lipid

Table 3. Parameters for brown adipocytes from guinea pigs chronically infused with isoprenaline, phenylephrine or both in combination

For details see the Experimental section. Values represent means $\pm$ S.E.M. for six isoprenaline-, five phenylephrine- and six phenylephrine/isoprenaline-infused animals. Control values are from Tables 1 and 2. The ratio noradrenaline/FCCP represents the ratio of the adipocyte respiration after acute noradrenaline treatment to that after optimal FCCP treatment (see Table 2).

	(Control)	Isoprenaline	Phenylephrine	Phenylephrine + isoprenaline
Wet wt. (g)	(3.33)	2.4 $\pm$ 0.1	2.5 $\pm$ 0.3	2.1 $\pm$ 0.3
Cytochrome oxidase (units/g)	(35)	32.6 $\pm$ 4.9	29.6 $\pm$ 6.2	113.0 $\pm$ 30.6
Cytochrome oxidase (total)	(113)	82.2 $\pm$ 8.0	83.6 $\pm$ 12.0	254.0 $\pm$ 74.0
Noradrenaline/FCCP	(0.21)	0.26 $\pm$ 0.04	0.17 $\pm$ 0.04	0.80 $\pm$ 0.08

mobilization closely matches that seen in the cold-adapted animals, as does the increase in cell number and the ability of the cells to express their full uncontrolled respiration (indicative of optimal uncoupling protein per mitochondrion). The only parameters that do not approach those of the cold-adapted animals are related to mitochondriogenesis. The total mitochondria increase by only 33% of that for cold-adaptation, and the respiratory responses per cell (Table 2) are similarly limited. This could suggest that an additional hormonal signal may be required for optimal mitochondrial proliferation.

β -Agonists cause significant hyperplasia in the rat (Heick *et al.*, 1973; Mory *et al.*, 1980). Our failure to reproduce this effect in the guinea pig may be due to a lesser role of brown-adipocyte hyperplasia in this animal (Rafael *et al.*, 1986) compared with the rat (Bukowiecki *et al.*, 1982).

Under our conditions, chronic β -adrenergic stimulation by isoprenaline (Table 3) fails to produce thermogenically competent cells, in contrast with noradrenaline (Tables 1 and 2). In the literature, the competency of β -adrenergic stimulation to induce uncoupling protein is ambiguous. On the one hand, chronic isoprenaline treatment is unable to increase the mitochondrial complement of uncoupling protein in rats (Mory *et al.*, 1980) and mice (Young *et al.*, 1984). However, an acute increase in GDP-binding capacity ('unmasking') has been reported after a single injection of β -agonist in the rat (Bryant *et al.*, 1984). Furthermore, Ricquier *et al.* (1986) reported an increase within 15 min in the nascent uncoupling protein transcripts in nuclei from rat brown adipose tissue after administration of the β -agonist BRL 26830A *in vivo*. Finally, an increase in uncoupling-protein mRNA was apparent after 48 h of drug administration, indicating that transcription of the gene encoding the uncoupling protein is under β -adrenergic control.

Our test for thermogenic competence (the ability of acute noradrenaline treatment to evoke fully uncontrolled respiration) is a very stringent criterion. It is possible that a pure chronic β -adrenergic agonist can induce uncoupling protein, but that, in the absence of a parallel α -adrenergic stimulus, any uncoupling protein does not become functional in the mitochondrial inner membrane. Blood flow to brown adipose tissue is under α -adrenergic control (Ma & Foster, 1984). It is conceivable therefore that the α -adrenergic requirement may reflect a need for a chronically increased blood flow rather than reflecting a receptor on the brown adipocyte itself.

S.A.C. was supported by a grant from the Scottish Home and Health Department.

REFERENCES

- Arch, J. R. S., Ainsworth, A. T., Cawthorne, M. A., Piercy, V., Sennit, M. V., Thody, V. E., Wilson, C. & Wilson, S. (1984) *Nature* (London) **309**, 163–165
- Brück, K. (1970) in *Brown Adipose Tissue* (Lindberg, O., ed.), pp. 117–154, American Elsevier, New York
- Bryant, K. R., Rothwell, N. J. & Stock, M. J. (1984) *Biosci. Rep.* **4**, 523–533
- Bukowiecki, L., Follea, N., Paradis, A. & Collet, A. (1980) *Am. J. Physiol.* **238**, E552–E563
- Bukowiecki, L. J., Collet, A. J., Follea, N., Guay, G. & Jahjah, L. (1982) *Am. J. Physiol.* **242**, E353–E359
- Cunningham, S. A., Wiesinger, H. & Nicholls, D. G. (1986) *Eur. J. Biochem.* **157**, 415–420
- Desautels, M. & Himms-Hagen, J. (1979) *Can. J. Biochem.* **57**, 968–976
- Dominguez, M. J., Fernandez, M., Elliot, K. & Benito, M. (1986) *Biochem. Biophys. Res. Commun.* **138**, 1390–1394
- Fain, J. N., Mohell, N., Wallace, M. A. & Mills, I. (1984) *Metab. Clin. Exp.* **33**, 289–294
- Garcia-Sainz, J. A., Hasler, A. K. & Fain, J. N. (1980) *Biochem. Pharmacol.* **29**, 3330–3333
- Heick, H. M. C., Vachon, C., Kallai, M. A., Begin-Heick, N. & LeBlanc, J. (1973) *Can. J. Physiol. Pharmacol.* **51**, 751–758
- LeBlanc, J. A. & Villemare, A. (1970) *Am. J. Physiol.* **218**, 1742–1745
- Locke, R. M., Rial, E., Scott, I. D. & Nicholls, D. G. (1982a) *Eur. J. Biochem.* **129**, 373–380
- Locke, R. M., Rial, E. & Nicholls, D. G. (1982b) *Eur. J. Biochem.* **129**, 381–387
- Ma, S. W. Y. & Foster, D. O. (1984) *Can. J. Physiol. Pharmacol.* **62**, 943–948
- Mohell, N., Nedergaard, J. & Cannon, B. (1983) *Eur. J. Pharmacol.* **93**, 183–186
- Mory, G., Ricquier, D. & Hemon, P. (1980) *J. Physiol. (Paris)* **76**, 859–864
- Mory, G., Bouillaud, F., Combe-George, M. & Ricquier, D. (1984) *FEBS Lett.* **166**, 393–396
- Nedergaard, J. & Cannon, B. (1984) in *Bioenergetics* (Ernster, L., ed.), pp. 291–314. Elsevier
- Nicholls, D. G. (1976) *Eur. J. Biochem.* **49**, 585–593
- Nicholls, D. G. & Locke, R. M. (1984) *Physiol. Rev.* **64**, 1–64
- Nicholls, D. G., Cunningham, S. A. & Rial, E. (1986) in *Brown Adipose Tissue* (Trayhurn, P. & Nicholls, D. G., eds.), pp. 52–85, Edward Arnold, London
- Raasmaja, A., Mohell, N. & Nedergaard, J. (1985) *Eur. J. Pharmacol.* **106**, 489–498
- Rafael, J. (1983) in *Methods in Enzymatic Analysis*, vol. 3 (Bergmeyer, H. U., ed.), pp. 266–263, Verlag Chemie, Weinheim

- Rafael, J., Fesser, W. & Nicholls, D. G. (1986) *Am. J. Physiol.* **250**, C1–C9
- Rial, E. & Nicholls, D. G. (1984) *Biochem. J.* **222**, 685–693
- Rial, E., Poustie, A. & Nicholls, D. G. (1983) *Eur. J. Biochem.* **137**, 197–203
- Ricquier, D., Mory, G., Nechad, M., Combes-George, M. & Thibault, J. (1983) *Am. J. Physiol.* **245**, C172–C177
- Ricquier, D., Mory, G., Bouillaud, F., Combes-George, M. & Thibault, J. (1985) *Reprod. Nutr. Dev.* **25**, 175–181
- Ricquier, D., Bouillaud, F., Toumelin, P., Mory, G., Bazin, R., Arch, J. & Penicaud, L. (1986) *J. Biol. Chem.* **261**, 13905–13910
- Schimmel, R., McCarthy, L. & McHahon, K. (1983) *Am. J. Physiol.* **244**, C362–C368
- Trayhurn, P. & Nicholls, D. G. (1986) *Brown Adipose Tissue*, Edward Arnold, London
- Wang, C. S. & Smith, R. L. (1975) *Anal. Biochem.* **63**, 414–417
- Young, P., Cawthorne, M. A., Levy, A. L. & Wilson, K. (1984) *FEBS Lett.* **176**, 16–20

Received 28 November 1986/18 March 1987; accepted 2 April 1987