The mitochondrial uncoupling protein from guinea-pig brown adipose tissue

Synchronous increase in structural and functional parameters during cold-adaptation

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(Received 26 March 1984/Accepted 30 May 1984)

The time-course for the induction of the uncoupling pathway in the inner membrane of brown-fat mitochondria from cold-adapting guinea pigs was studied. The amount of the protein was quantified from the capacity for high-affinity binding of GDP to the intact mitochondria, and was compared with the functional parameters diagnostic of the protein, namely the nucleotide-sensitive proton conductance and the sensitivity to uncoupling by low concentrations of fatty acids. A monophasic increase in nucleotide titre was observed, with no evidence of an early 'unmasking' of pre-existing nucleotide-binding sites. The nucleotide-sensitive conductance increased in precise synchrony with the nucleotide-binding capacity. Mitochondria from newborn animals, and those from acutely cold-adapted animals, showed anomalously low sensitivities to uncoupling by fatty acids.

The inner membrane of mitochondria from thermogenically active brown fat possesses a tissue-specific 32kDa integral protein (Heaton *et al.*, 1978), which functions as a regulatable proton short-circuit capable of uncoupling respiration from ATP synthesis (reviewed in Nicholls, 1979; Nicholls & Locke, 1984). The protein displays a high proton conductance, which can be inhibited *in vitro* by the binding of purine nucleotides (Nicholls, 1974). Extremely low concentrations of non-esterified fatty acids over-ride this low-conductance state (Locke *et al.*, 1982a,b; Rial *et al.*, 1983), consistent with fatty acids being the acute physiological regulators of the conductance.

The uncoupling protein, quantified by purine nucleotide binding (Nicholls, 1976), is induced at birth in the guinea pig, rapidly regresses when the animals are reared at thermoneutral temperature, and increases again when the animals are cold-adapted (Rafael & Heldt, 1976; Heaton *et al.*, 1978). This facultative expression is also seen in other rodents during development, cold-adaptation, hyperphagia and in cases of genetic obesity

(review in Nicholls & Locke, 1984), and has been studied additionally by densitometric analysis of polyacrylamide gels (Ricquier & Kader, 1976), or by radioimmunoassay (Cannon *et al.*, 1982; Ricquier *et al.*, 1983; Lean *et al.*, 1983).

The only animal in which the time-course of induction of the protein has been followed in any detail is the rat. The laboratory of Himms-Hagen (Desautels *et al.*, 1978; Desautels & Himms-Hagen, 1979, 1980; Himms-Hagen *et al.*, 1980) reported a marked lack of synchrony in two parameters that both apparently quantify the protein: a rapid increase in GDP binding was observed in the first hours of cold-adaptation, but this was unaccompanied by a detectable increase in the 30–35kDa range of proteins quantified by SDS/polyacrylamide-gel electrophoresis. To account for this anomaly, they have proposed that pre-existing, but latent, uncoupling protein in the mitochondrial inner membranes becomes 'unmasked' during the first few hours of cold-exposure, revealing additional nucleotide-binding sites without the necessity for protein synthesis.

There have been no studies in which the time-course of induction of functional parameters, such as the high proton conductance sensitive to purine nucleotides and fatty acids (Nicholls, 1974), has been correlated with the increase in the uncoupling protein. Without such a comparison it is speculative to assume that an increase in the

Abbreviations used; TPP⁺, tetraphenylphosphonium cation; Tes, 2-[[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-amino]ethanesulphonate; $\Delta\psi$, mitochondrial membrane potential; *JO*, proton current across the mitochondrial inner membrane; $C_m(H^+)$, effective proton conductance of the membrane; SDS, sodium dodecyl sulphate.

number of nucleotide-binding sites is synonymous with an increase in the proton conductance of the membrane, or in the sensitivity to uncoupling by fatty acids.

We conclude that the induction of the uncoupling protein in the guinea-pig mitochondrion follows a simple monotonic time-course, with a half-time of 76 h; therefore there is no evidence of any anomalous rapid 'unmasking' of nucleotide-binding sites. The nucleotide-binding capacity, nucleotide-sensitive proton conductance and fatty acid sensitivity all increase synchronously during cold-adaptation.

Materials and methods

Experimental animals

Dunkin-Hartley strain guinea pigs were kept from birth in a warm cabinet at $28 \pm 2^\circ\text{C}$ to ensure thermoneutrality (Brück, 1970). Littermates were killed at 3 weeks after either being maintained at thermoneutral temperature as 'warm controls', or after having been transferred to a cold-chamber ($6 \pm 2^\circ\text{C}$) for 17 h, 48 h or 96 h before being killed. Fully cold-adapted animals were 5-week-old guinea pigs that had been maintained in the cold environment for 18 days before being killed. The newborn animals studied were less than 12 h old. Animals had a 12 h-light/12 h-dark regimen and were allowed free access to water and a pellet diet containing (w/w) 19.5% protein, 46.9% carbohydrate, 3.5% fat and 10.4% fibre (diet FD1, from Special Diets Service, Witham, Essex, U.K.).

Mitochondrial preparation

Brown adipose tissue was obtained from interscapular, dorsal, perirenal and spinal-cord sites. After dissection, the approximate density of the tissue was estimated from the displacement of medium by a known mass of submerged tissue in a measuring cylinder. Mitochondria were prepared as previously described (Nicholls, 1974), but with 80 μM -albumin present in all preparation media, except for the final centrifugation and resuspension in 100 mM-KCl/5 mM-Tes (sodium salt), pH 7.4.

Determination of membrane potential, respiration and proton conductance

A 1.7 ml-capacity thermostatically controlled chamber with oxygen- and TPP^+ -selective electrodes was used as previously described (Locke *et al.*, 1982a). Mitochondria (0.3 mg/ml) were incubated at 30°C in a medium containing: 50 mM-KCl, 10 mM-Tes (sodium salt), pH 7.0, 1 mM-sodium phosphate, 2 mM- MgCl_2 , 5 mM-sodium pyruvate, 3 mM-sodium malate, 5 mM-sodium α -glycerophosphate, 1 μg of oligomycin/ml and 5 μM -

TPP^+ . Further additions are described in the legends.

On the assumption that $^{86}\text{Rb}^+$ distributes ideally without binding, the extent to which the $[^3\text{H}]\text{TPP}^+$ accumulation ratio overestimates the membrane potential can be estimated by comparing the accumulation ratios of the two cations in the same experiment, when membrane potential was varied by the addition of various concentrations of KCl in the presence of valinomycin. The incubation medium contained 10 mM-Tes (sodium salt), pH 7.0, 5 mM-sodium phosphate, 2 mM- MgCl_2 , 3 mM-GDP (sodium salt), 5 mM-sodium α -glycerophosphate, 5 mM-sodium pyruvate, 3 mM-sodium malate, 0.5 μM -valinomycin, 1 mM- $[^{14}\text{C}]$ -sucrose (0.18 $\mu\text{Ci/ml}$), 5 μM - $[^3\text{H}]\text{TPP}^+$ (0.14 $\mu\text{Ci/ml}$), 50 μM - $^{86}\text{RbCl}$ (0.3 $\mu\text{Ci/ml}$) together with KCl concentrations (0–20 mM). NaCl was added so that the sum of KCl and NaCl was 50 mM. Mitochondria (0.3 mg of protein/ml of incubation mixture) were incubated in this medium for 2.5 min, after which samples were taken into Eppendorf tubes containing silicone oil and centrifuged in an Eppendorf model 5412 bench microcentrifuge at full speed for 60 s. The pellets were resuspended in 20 μl of 5% (v/v) SDS and transferred into vials for radioactivity counting with 5 ml of Scintran Cocktail T (BDH). Over the range of TPP^+ -indicated membrane potential in excess of 160 mV the membrane potential indicated by TPP^+ was found to be related to that of $^{86}\text{Rb}^+$ by the relationship:

$$\Delta\psi_{\text{TPP}^+} = 1.2\Delta\psi_{\text{Rb}^+} - 54 \text{ mV}$$

The effective proton conductance of the mitochondrial inner membrane was estimated from the corrected membrane potential ($\Delta\psi$) and the respiratory rate (JO), assuming an average H^+/O stoichiometry of 7 for the mixed oxidation of α -glycerophosphate and pyruvate plus malate (Locke *et al.*, 1982a). The pH gradient (which should be small in the presence of 5 mM-phosphate) was ignored, and membrane potential was taken to be synonymous with proton electrochemical potential. Conductance ($\text{nmol of H}^+ \cdot \text{min}^{-1} \cdot \text{mg of protein}^{-1} \cdot \text{mV}^{-1}$) was therefore calculated from the relationship:

$$C_m(\text{H}^+) = 7JO/\Delta\psi$$

$[^3\text{H}]\text{GDP}$ binding

The specific activity of nucleotide-binding sites on the mitochondria was determined in a medium containing 100 mM-potassium acetate, 5 mM-Tes (sodium salt), 16 μM -albumin, 2 μM -rotenone, 0.4 μCi of $[^{14}\text{C}]$ -sucrose/ml and five different concentrations (2–10 μM) of $[^3\text{H}]\text{GDP}$ (sodium salt, 1.1 $\mu\text{Ci/ml}$). After 5 min at 30°C , duplicate 250 μl samples of mitochondria were separated by

centrifugation as described previously (Rial & Nicholls, 1983). Scatchard analysis of the results was performed with the Rothamsted Maximum Likelihood Program (Ross, 1978).

Other assays

Cytochrome *c*:O₂ oxidoreductase activity (cytochrome *c* oxidase, EC 1.9.3.1) was determined spectrophotometrically by the method of Sottocasa *et al.* (1967). Mitochondrial protein was determined by the biuret method, with albumin as standard.

Materials

Radioisotopes were obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). TPP⁺ was from Fluorochem Ltd. (Glossop, Derbyshire, U.K.). Other reagents were from Sigma Chemical Co. (Poole, Dorset, U.K.).

Results

In preliminary studies it was found that guinea pigs maintained at normal room temperature (20–23°C) were significantly cold-adapted, as judged from the GDP-binding titre of their mitochondria. Animals were therefore maintained from birth at 28 ± 2°C to ensure thermoneutrality (Brück, 1970).

Table 1 summarizes the effects of cold-adaptation on the animals and on the composition of their brown adipose tissue and its mitochondria. The animals undergo a rapid weight loss during the first 2 days of cold exposure. This is also reflected in the wet weight of the recovered brown adipose tissue. The decrease almost certainly reflects a loss of triacylglycerols, since the density of the tissue increases rapidly. Over the first 4 days of cold-adaptation there is no general increase in the amount of mitochondria in the tissue (Fig. 1). The total protein and total cytochrome oxidase activity of the recovered mitochondria do not change significantly over this period, any increase per g

Fig. 1. Purine-nucleotide-binding capacity and mitochondrial protein recovery from the brown adipose tissue of newborn and cold-adapting guinea pigs (a) GDP binding per mg of mitochondrial protein determined by Scatchard analysis as described in the Materials and methods section. (b) Total brown-fat mitochondrial protein recovered per animal. (c) Total GDP-binding capacity of recovered mitochondria. Symbols: nb, newborn animals; wa, warm-adapted animals; 17h, 2d, 4d, 18d; animals cold-adapted for 17h, 2 days, 4 days and 18 days respectively. Error bars represent the S.E.M. for at least five determinations.

Table 1. General parameters of the newborn and cold-adapting guinea pigs employed in the study

Conditions	Animals			Brown adipose tissue		Brown-adipose-tissue mitochondria	
	No.	Age (days)	Weight (g)	Wet wt. (g)	Density (g/ml)	Protein (mg/g of tissue)	Cytochrome oxidase total activity (units)
Newborn	6	<12h	94 ± 7	3.2 ± 0.3	0.96 ± 0.01	2.8 ± 0.3	2.15 ± 0.28
Warm (28°C)	5	21–23	305 ± 15	7.4 ± 0.8	0.93 ± 0.02	1.1 ± 0.2	1.85 ± 0.53
Cold-adapted (6°C)							
17h	6	21–23	268 ± 14	5.4 ± 0.4	0.98 ± 0.01	1.3 ± 0.2	1.96 ± 0.34
48h	5	21–23	219 ± 7	2.3 ± 0.1	0.99 ± 0.01	3.8 ± 0.5	1.27 ± 0.55
96h	5	21–23	229 ± 5	1.7 ± 0.3	–	5.3 ± 0.5	1.69 ± 0.57
18 days	6	32–38	310 ± 25	3.7 ± 0.6	1.02 ± 0.02	8.4 ± 1.2	4.10 ± 0.87

wet weight being accounted for by the loss of triacylglycerols (Table 1).

The high-affinity binding of [^3H]GDP to brown-fat mitochondria appears to be almost entirely limited to the uncoupling protein (Rial & Nicholls, 1983) and is therefore a valid means of quantifying the protein as long as a Scatchard analysis is performed to estimate maximal binding capacity and eliminate non-functional low-affinity binding sites. Fig. 1 shows that there is an increase in binding capacity either expressed per mg of mitochondrial protein or extrapolated to the total recovered tissue. This increase is already apparent in the 17h cold-exposed animals. Although the binding capacity increased, there was no significant change in the K_d of the high-affinity binding

site, which remained at $2.3 \pm 0.18 \mu\text{M}$ (31 determinations).

Although we could detect a slight increase in GDP binding by 17h, we saw nothing comparable with the 5-fold increase per mg of mitochondrial protein seen in the rat by Desautels *et al.* (1978). Those authors found that more than 50% of the final increase in the specific activity of GDP binding was complete within 12h. In contrast, we found with the guinea pig that, if we take the nucleotide binding per mg of mitochondrial protein after 18 days of cold-exposure to be 100%, the amount of the uncoupling protein in the mitochondria from the warm-adapted animal would be 13%, increasing only to 19% after 17h, 37% after 48h and 59% after 96h (Fig. 1).

Fig. 2. Representative respiration and membrane-potential traces for brown-fat mitochondria from (i) warm-adapted and (ii) 18-day cold-adapted guinea pigs

Membrane potentials were calculated after allowing for binding of TPP^+ as described in the Materials and methods section. Experiments are shown to illustrate the basis for the calculation of the parameters shown in subsequent Figures. Additions: a, mitochondria (0.3 mg of protein/ml of incubation); b, $16 \mu\text{M}$ -albumin; c, 3 mM -GDP; d and e, $16 \mu\text{M}$ -palmitate. The rate of uncontrolled respiration (Fig. 4) was determined in the absence of both albumin and nucleotide (i.e. following addition 'a').

In terms of the total recovery of the uncoupling protein from the tissue, the warm-adapted animal has only 3.4% of the final tissue content of uncoupling protein, and this increases to only 4.3% during the first 17 h. Guinea pigs therefore do not show any indication of the rapid 'unmasking' of binding sites reported for the rat (Desautels *et al.*, 1978).

Development of regulatable proton conductance

The brown-fat mitochondria from cold-adapted guinea pigs differ from those isolated from warm-adapted controls not only because of their greater capacity to bind GDP, but also because they possess a high proton conductance, which is inhibited as the purine nucleotides bind to the protein (Nicholls, 1974; Rial & Nicholls, 1983). Submicromolar concentrations of non-esterified fatty acids can override the low-conductance state induced by nucleotides in the cold-adapted mito-

chondria, but are much less effective in uncoupling those lacking the uncoupling protein (Locke *et al.*, 1982a,b).

Figs. 2 and 3 show the principles of the approach that we have taken to determine, in a single experiment, the open-channel conductance of the uncoupling protein, its sensitivity to purine nucleotide, the residual conductance of the membrane, and the sensitivity with which fatty acids reverse the low-conductance state. The use of the combination of an oxygen electrode and a TPP^+ -selective electrode to monitor mitochondrial respiration and membrane potential, and hence to estimate the effective proton conductance of the membrane, has been described previously (Locke *et al.*, 1982a). In Fig. 2, two extreme conditions are shown for illustration, warm-adapted and 18 days-cold-adapted. The mitochondria were added to a medium containing α -glycerophosphate, pyruvate and malate to ensure a maximal supply of electrons

Fig. 3. Calculation of membrane proton conductances from the electrode traces in Fig. 2

Conductances were calculated from the membrane potentials and respiratory rates in Fig. 2 as described in the Materials and methods section. Additions b–e correspond to those in Fig. 2, mitochondria from warm-adapted (i) and cold-adapted (ii) animals being added at zero time. The conductances before the addition of albumin were too high to be accurately determined. Residual conductance, R, was determined in the presence of GDP and albumin. GDP-sensitive conductance, G, was defined as the difference between the residual conductance and the conductance in the presence of albumin alone. Fatty-acid-induced conductance, F, was arbitrarily defined as the difference between the residual conductance and that after the second addition of palmitate.

to the respiratory chain. The incubation medium initially lacks purine nucleotides, with the result that the uncoupling protein is in a high-conductance state, even after albumin is added to remove residual endogenous fatty acids (Nicholls, 1974). Addition of sufficient purine nucleotide to saturate the high-affinity binding site on the uncoupling protein inhibits the conductance of the pathway (Nicholls, 1974). The extent of the conductance decrease is 4.4 and 26 nmol of $\text{H}^+ \cdot \text{min}^{-1} \cdot \text{mg}$ of protein $^{-1} \cdot \text{mV}^{-1}$ respectively for the warm- and cold-adapted animals respectively. The decrement on adding nucleotide will be used to quantify the capacity of the uncoupling protein to conduct protons.

Fatty acids over-ride the low conductance state imposed on the protein by the binding of purine nucleotide (Locke *et al.*, 1982a,b; Rial *et al.*, 1983). To quantify the sensitivity of the proton conductance to fatty acids, palmitate was added to give molar ratios of palmitate/albumin of 1:1 and 2:1. As previously shown (Locke *et al.*, 1982b), the fatty acid is much more effective in increasing the conductance of the mitochondria from the cold-adapted animal, i.e. the one possessing the uncoupling protein.

The capacity for uncontrolled respiration in the warm-adapted animal is the same as in the newborn, and only 30% below that of the fully cold-adapted animal; whereas, in contrast, the nucleotide-sensitive proton conductance increases by 460% over the same period (Fig. 4). If the GDP-sensitive conductance is plotted as a function of the GDP-binding capacity, a linear relationship is obtained, with $r = 0.990$ (Fig. 5). The newborn animal also fits to the same relationship. In other

words, we observe a precise synchrony between the appearance of new binding sites and the conductance diagnostic of the functional uncoupling protein.

The greater sensitivity to uncoupling by fatty acids of mitochondria containing the uncoupling protein has provided the basis of our contention that non-esterified fatty acids provide the acute physiological regulator of proton conductance in brown adipose tissue *in situ* (Locke *et al.*, 1982a,b; Rial *et al.*, 1983). Fig. 6 shows that the ability of a

Fig. 5. Correlation between GDP-sensitive proton conductance and GDP-binding capacity

Data and abbreviations are taken from Figs. 1 and 4. Error bars are shown where they exceed the dimensions of the symbols.

Fig. 4. GDP-sensitive proton conductance (●) and the rate of uncontrolled respiration (○) in mitochondria from newborn, warm-adapted and cold-adapting guinea pigs. Parameters were calculated from experiments similar to those depicted in Fig. 2. The abbreviations for the adaptive state of the animals are as in Fig. 1. Each point represents the mean $\pm$ S.E.M. for at least five experiments.

Fig. 6. Correlation between fatty-acid-induced proton conductance and GDP-binding capacity

Fatty-acid-induced proton conductance is defined as in Fig. 3. GDP-binding capacity and abbreviations are taken from Fig. 1.

Fig. 7. Residual proton conductance of mitochondria from newborn, warm-adapted and cold-adapting guinea pigs. Residual proton conductance was as defined in Fig. 3. Note that the scale of conductance differs from that in Fig. 4. Abbreviations are as for Fig. 1.

2:1 molar ratio of palmitate to albumin to override the low-conductance state induced by GDP correlates with the nucleotide-binding capacity, but with two exceptions. Firstly, the sensitivity actually falls during the first stages of cold-adaptation, and secondly the newborn animal provides mitochondria that are markedly insensitive to uncoupling by fatty acids. A linear relationship ($r = 0.999$) between the two parameters is only seen for the animals in the process of cold-adaptation, but not for the mitochondria from the newborn and warm-adapted guinea-pigs.

The residual proton conductance of the membrane (when the purine-nucleotide-binding site is saturated and fatty acids are absent) also shows an unexpected variation during cold-adaptation (Fig. 7). The fully cold-adapted mitochondria consistently demonstrate a low residual conductance, equal to some 50% of that of the warm-adapted control. However, during the period of cold-adaptation the residual conductance initially increases before attaining this low value. Finally the mitochondria from the newborn show a high residual proton conductance. It may be significant that the conditions where residual conductance is highest are those which show a lower fatty-acid-sensitivity.

Discussion

Latent binding sites

The present results demonstrate that 'unmasking' (the appearance of a large number of extra nucleotide-binding sites on the rat brown-fat mitochondrial inner membrane within the first hours of cold-adaptation; Desautels *et al.*, 1978) is not a general phenomenon of cold-adaptation, since it is completely absent from the cold-adapting guinea pig. Instead, the density of

binding sites on the mitochondrial membrane (expressed in relation to mitochondrial protein) increases steadily over the period of adaptation, with the half-maximal increase in binding capacity only being achieved after some 3 days (Fig. 1). This is in great contrast with the rat, where some 80% of the increase in nucleotide binding seen with the 2-4-weeks cold-adapted animal has been reported to be already apparent by 12 h (Desautels *et al.*, 1978).

Three lines of evidence led Desautels *et al.* (1978) and Desautels & Himms-Hagen (1980) to propose that the initial rapid increase in GDP titre seen in the rat was not due to protein synthesis *de novo*, but rather to an exposure, or unmasking, of pre-existing nucleotide-binding sites by a conformational change brought about by the initiation of the cold stress. Firstly, the very rapidity of the reported increase did not seem consistent with a process requiring protein synthesis. Secondly, the increase in GDP binding was in contrast with the relatively slow increase in the size of the 32 kDa region of the polyacrylamide-gel traces (Desautels *et al.*, 1978), and thirdly, the initial increase in binding was only partially sensitive to cycloheximide (Himms-Hagen *et al.*, 1980).

In fact, the time course with which the 32 kDa-protein band increases in gels of rat brown-fat mitochondria (Desautels *et al.*, 1978) fits well with our time course of binding increase in the guinea pig (Fig. 1). Although we have not attempted here to quantify the uncoupling protein by either densitometric analysis of gels or radioimmunoassay, a study by Ashwell *et al.* (1983) on the mouse has shown that, at the end of 3 weeks adaptation to various lowered environmental temperatures, the amount of uncoupling protein assayed by antibody correlates closely with the extent of binding of $10 \mu\text{M}$ -GDP to the mitochondria. We have no reason, therefore, to doubt that the steady increase in nucleotide-binding capacity, measured from Scatchard plots in the present paper, represents anything other than a true increase in incorporated uncoupling protein.

This contention is strengthened by our finding that the primary parameter diagnostic of functional uncoupling protein, the nucleotide-sensitive proton conductance of the membrane (Nicholls, 1974), increases precisely in parallel with the increased GDP-binding titre. It appears therefore that cold-adaptation is associated with a straightforward monotonic incorporation of functional uncoupling protein into the mitochondrial inner membrane, and that any complexities involving conformational changes which reveal pre-existing, latent, nucleotide-binding sites (Desautels *et al.*, 1978; Himms-Hagen *et al.*, 1980; Desautels & Himms-Hagen, 1979, 1980) do not apply to the cold-adapting guinea pig.

Time course of uncoupling-protein synthesis

The 18-day-cold adapted guinea pig shows a 5-fold increase in the recovered yield of brown-fat mitochondrial protein, whereas the specific activity of the uncoupling protein increases 8-fold within the mitochondrial membrane. Thus there is an overall 40-fold increase in the amount of uncoupling protein in the animal. In the mouse an even more spectacular increase has been recorded by radioimmunoassay, since an 80-fold increment is seen when animals maximally cold-stressed to -2°C are compared with those maintained at thermoneutrality (Ashwell *et al.*, 1983).

The cold-induced increase in uncoupling protein occurs with a very different time course from that for general mitochondrial protein synthesis. During the first 4 days of cold-adaptation, there is no change in the recoverable mitochondrial protein, while the specific activity of the uncoupling protein increases 4-fold (Fig. 1). This suggests that the protein is being incorporated into pre-existing brown-fat mitochondria, rather than there being an initial synthesis *de novo* of mitochondria containing a full complement of the protein.

Correlation between structural and functional parameters

The present study is the first in which a systematic attempt has been made to correlate the changes in amount of the uncoupling protein with the functional parameters which are associated with the protein in the fully cold-adapted state. Without such direct evidence it is presumptive to assume that an increase in GDP-binding titre is automatically associated with an increase in proton-conductance capacity, particularly in view of the apparent lack of synchrony in the structural parameters discussed above for the rat.

There is no doubt that the transfer of 3-week-old newly weaned guinea pigs from the thermoneutral cabinet at 28°C to a cold-room at 6°C imposes a severe cold-stress. The animals lose 28% of their body weight within 2 days, and the recovered brown fat is decreased in wet weight and increased in density, consistent with an extensive depletion of triacylglycerols. This weight loss and triacylglycerol depletion of this tissue has been recorded by Ricquier *et al.* (1979) and appears to be a particular feature of the guinea pig, since in other rodents hypertrophy seems dominant (Ricquier *et al.*, 1979).

The most accurate way to quantify the function of the uncoupling protein is in terms of its effective proton conductance, the proton current carried across the membrane per unit of proton electrochemical potential. The decrease in conductance on adding a saturating concentration of purine

nucleotide to fatty-acid-depleted mitochondria correlates extremely well with the capacity of the nucleotide-binding site during cold-adaptation (Fig. 5). This validates what has previously merely been an assumption, that GDP-binding capacity is an accurate criterion of the capacity of the uncoupling pathway during the stages of cold-adaptation.

It would now clearly be of value to establish whether the same correspondence is seen for the cold-adapting rat, i.e. is there a sudden increase in the nucleotide-sensitive proton conductance of the brown-fat mitochondria in the first hours of cold-adaptation that correlates with the unmasking of binding sites?

The proposal that fatty acids are the physiological regulators of the uncoupling protein is based on the correlation between the presence of the protein and the ability of long-chain fatty acids to over-ride the nucleotide-induced low-conductance state (Locke *et al.*, 1982*a,b*). In the present paper we show that this correlation holds during cold-adaptation, but that the mitochondria from the newborn guinea pig are anomalous, with a high GDP titre and nucleotide-sensitive conductance, but a low sensitivity to fatty acids, and an anomalously high basal proton conductance.

To a lesser extent the mitochondria from the 17 h-cold-stressed animal are also anomalous, since their sensitivity to fatty acids is significantly decreased relative to the 'warm control' and their basal conductance is enhanced. These two conditions (at birth and during the first hours of cold-stress) are those where the thermogenic demand on the tissue is the most intense, and the significance of this variation from the expected behaviour remains to be established.

This work was supported in part by a project grant from the M.R.C. E. R. was supported by a grant from the Spanish 'Dirección General de Política Científica'. The expert assistance of Mr. Craig Adam, Mr. Stephen Wilkie and Miss Elizabeth Emslie is gratefully acknowledged.

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