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Regulation of the level of uncoupling protein in brown adipose tissue by insulin requires the mediation of the sympathetic nervous system

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The role of the sympathetic nervous system in the regulation by insulin of the level of uncoupling protein in brown adipose tissue has been examined. The amount of uncoupling protein was substantially reduced in streptozotocin-diabetic rats, while insulin replacement to diabetic animals induced a partial restoration. Unilateral denervation of the interscapular brown fat pads also lowered the amount of uncoupling protein, and in diabetic animals inhibited the stimulation of the level of the protein by insulin replacement. Maintenance of normal uncoupling protein levels requires both insulin and the sympathetic system; regulation of the protein by insulin involves sympathetic mediation.

Brown adipose tissue; Insulin; Uncoupling protein; Diabetes; Sympathetic nervous system; Noradrenaline

1. INTRODUCTION

Brown adipose tissue (BAT) is highly specialized for the generation of heat, both for thermoregulation and in relation to the regulation of body lipid stores [1–3]. Heat is generated in BAT by a proton conductance pathway, which acts as a proton short-circuit across the mitochondria dissociating substrate oxidation from ATP synthesis [1]. The proton conductance of BAT mitochondria is regulated by a tissue-specific 'uncoupling protein', M_r 32000 [1]. The level and activity of this protein vary in accordance with the physiological requirements for thermogenesis, and the adaptive state of an animal (see [2,3]).

Insulin stimulates thermogenesis [3–5], and plays an important role in the regulation of the level of uncoupling protein in BAT [6,7]. The induction of diabetes with streptozotocin induces a substantial fall in the amount of uncoupling protein, while insulin replacement to diabetic animals leads to a dose-dependent restoration of the protein [7]. Noradrenaline from the sympathetic nervous system has a dominant role in the overall control of thermogenesis [1–5], and there is evidence that insulin stimulates thermogenesis through a central activation of the sympathetic system [4,5]. However, insulin receptors are present in BAT [8], and direct effects of the hormone on the brown adipocyte, such as the stimulation of glucose uptake and of lipogenesis, have been demonstrated [9–12].

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In the present study we have examined whether insulin regulates the level of uncoupling protein primarily by a direct interaction with BAT, or through the mediation of the sympathetic system. This has been done by determining the effects of surgically denervating one side of the interscapular BAT of rats made diabetic with streptozotocin, in the presence and absence of replacement insulin. Each of the two interscapular BAT pads receives its own innervation [13], enabling comparisons to be made between innervated and denervated halves of the tissue.

2. MATERIALS AND METHODS

2.1. *Animals and surgery*

Lister hooded rats (Rowett colony), weighing 175–200 g, were made diabetic by an intraperitoneal injection of streptozotocin (75 mg/kg body wt). The rats were housed singly in plastic cages, with free access to a commercial low fat/high carbohydrate diet. The animal room was maintained at 21 ± 1°C, with a 12 h light/12 h dark cycle (lights from 07.00 h).

After 12 days of diabetes the rats were anaesthetized with ether, and interscapular BAT surgically denervated on one side only; care was taken to ensure that the innervation remained intact on the other side. The rats were divided into 4 weight-matched groups, and on osmotic minipump (Model 2002; Alzet Corp., USA) implanted subcutaneously in the dorsal region [7]. The pumps were set to deliver either 0 (diabetic group), 8, 30, or 70 units of insulin/kg initial body wt per day. The insulin was Iletin II, U-500, purified pork insulin (Lilly, USA). Rats were killed after 12 days of insulin infusion, and interscapular BAT removed [7].

2.2. *Preparation of mitochondria and immunoassay of uncoupling protein*

The two interscapular pads were separated into denervated and innervated halves, and weighed. The innervated and denervated pads were then processed separately. The tissue was homogenized, and a

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sample removed for the measurement of cytochrome oxidase activity [14]. Mitochondria were prepared and mitochondrial protein measured as previously [14].

The uncoupling protein concentration in the mitochondria was measured by a dot immunobinding assay, employing a rabbit anti-(ground squirrel uncoupling protein) serum [15]. A purified rat uncoupling protein standard was used in the immunoassay [14].

2.3. *Statistical analysis*

The effect of diabetes and insulin replacement was evaluated by one-way analysis of variance; significant differences were identified with the Fisher protected least significant different post hoc test. Student's paired *t*-test was used to assess the statistical significance of differences between innervated and denervated halves of interscapular BAT.

3. RESULTS

The induction of experimental diabetes with streptozotocin resulted in a complete inhibition of weight gain in the rats, which was reversed in a dose-dependent manner by insulin replacement (Fig. 1A). The amount of interscapular BAT was reduced in diabetic rats, while a partial restoration occurred with insulin replacement (Fig. 1B). Interscapular BAT pad weight was significantly greater in denervated than in innervated tissue of control rats, and in diabetic rats with the two highest levels of insulin replacement (Fig. 1B).

Cytochrome oxidase activity was measured, and the tissue mitochondrial protein content determined from the total cytochrome oxidase activity and the specific activity of the enzyme in the mitochondria. Both cytochrome oxidase activity and the mitochondrial protein content of interscapular BAT were substantially reduced in the diabetic rats, while some recovery was observed with insulin replacement (Fig. 2A and B).

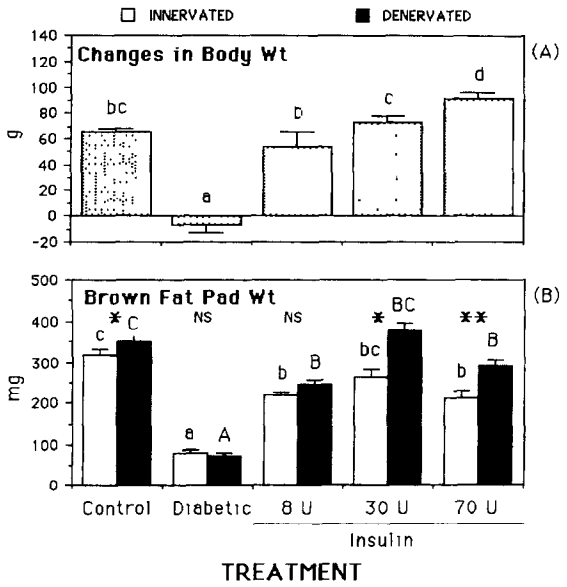

Fig. 1. Body weight gain (A) and interscapular brown adipose tissue pad weight (B) in diabetic rats, and rats with insulin replacement. For experimental details see text. Results are means ± SE, for 6–10 rats in each group. Values with different superscripts are significantly different (Fisher PLSD; $P < 0.05$). * $P < 0.05$, ** $P < 0.01$, compared with innervated pad in animals of the same group (Student's *t*-test). NS, not significant ($P > 0.05$).

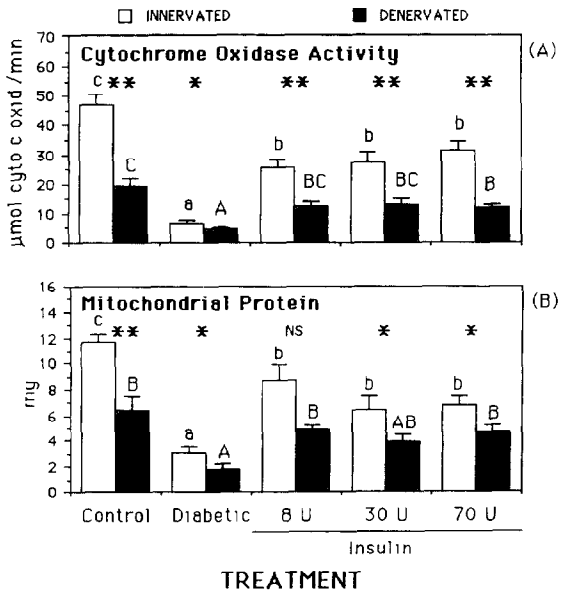

Fig. 2. Effect of unilateral denervation on cytochrome oxidase activity (A) and mitochondrial protein content (B) of interscapular brown adipose tissue pads of diabetic rats, and rats with insulin replacement. Results are means ± SE, for 6–10 rats in each group. Values with different superscripts are significantly different ($P < 0.05$). * $P < 0.05$, ** $P < 0.01$, compared with innervated pad in animals of the same group.

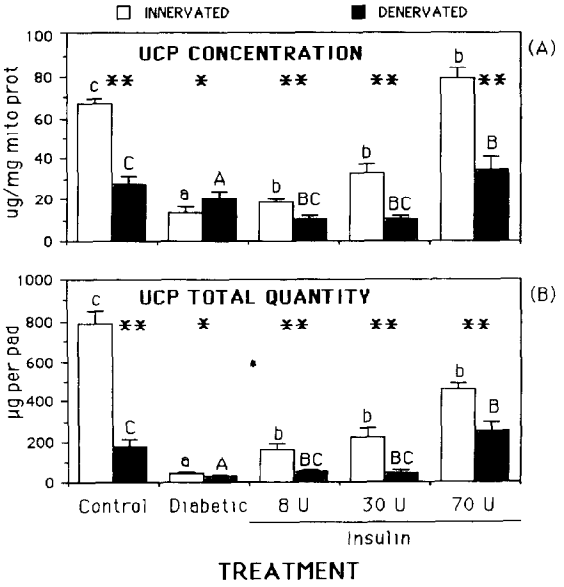

Fig. 3. Effect of unilateral denervation on the concentration of uncoupling protein (UCP) in mitochondria (A) and the total tissue uncoupling protein content (B) of interscapular brown adipose tissue pads of diabetic rats, and rats with insulin replacement. Results are means ± SE, for 6–10 rats in each group. Values with different superscripts are significantly different ($P < 0.05$). * $P < 0.05$, ** $P < 0.01$, compared with innervated pad in animals of the same group.

Both parameters were also significantly reduced by denervation in control and diabetic rats, and in diabetic animals with replacement insulin.

The specific mitochondrial concentration of uncoupling protein was substantially reduced in diabetic animals (Fig. 3A). The total amount of uncoupling protein in each interscapular pad, calculated from the specific mitochondrial concentration and the tissue mitochondrial protein content, was also greatly decreased in diabetes (Fig. 3B). Similar, though less substantial, effects were obtained by denervation alone (Fig. 3A and B). There was a partial restoration of the level of uncoupling protein (per mg of mitochondrial protein and per interscapular pad) with each dose of insulin replacement when the sympathetic innervation remained intact. However, only with the highest dose of insulin was there any substantive recovery of uncoupling protein in denervated BAT, although the amount of the protein remained significantly less than in innervated tissue (Fig. 3A and B).

4. DISCUSSION

The induction of diabetes with streptozotocin results in a substantial fall in the level of uncoupling protein in BAT of rats, with a consequent decrease in the capacity for thermogenesis. Insulin replacement to diabetic animals leads to a partial restoration of the amount of uncoupling protein. This suggests that insulin plays an important role in the regulation of uncoupling protein in BAT, in agreement with our previous studies on mice [7]. Rats were used in the present experiments because of the difficulty of performing selective denervation of interscapular BAT in animals as small as mice.

Denervation of one of the interscapular BAT pads of normal rats resulted in a marked fall in the level of uncoupling protein in that particular pad, relative to the contralateral pad in which the innervation remained intact. Denervation also inhibited the recovery of the amount of uncoupling protein in rats with different doses of insulin replacement. This clearly suggests that noradrenaline from the extensive sympathetic innervation to BAT is also important in the regulation of the amount of uncoupling protein, which is consistent with the central role attributed to the sympathetic system in the overall stimulation of thermogenesis [2–5]. The reduction in the total tissue content of uncoupling protein with denervation is a consequence of a decrease in the specific mitochondrial concentration of the protein, together with a fall in mitochondrial mass. The same combination of changes is also responsible for the large reduction in the amount of uncoupling protein in experimental diabetes.

It is concluded that both insulin and an intact sympathetic innervation are required for the maintenance of normal levels of uncoupling protein in BAT. This suggests that insulin regulates the level of the protein through an interaction with the sympathetic system. Sympathetic activity in BAT is reduced in streptozotocin-induced diabetes [16], and insulin is considered to stimulate the sympathetic system [4,5]. It seems likely, therefore, that the regulatory effects of insulin on uncoupling protein are mediated by a central stimulation of sympathetic activity to BAT. However, in view of recent evidence of synergistic effects between insulin and noradrenaline in isolated brown adipocytes [9–12], the possibility of a direct interaction of the two hormones at the cellular level in BAT cannot be excluded.

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