Effect of *E. Coli* Endotoxin on Temperature, Oxygen Consumption and Brown Adipose Tissue Thermogenesis in Rats and Mice

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Received June 3, 1987

KEY WORDS: endotoxin; brown adipose tissue; energy expenditure.

The effects of *E. coli* endotoxin 0127 B8 on oxygen consumption, temperature, and on the activity of the proton conductance pathway in brown adipose tissue (BAT) were investigated in rats and mice. In rats an increase was observed in rectal and skin temperature, whole body oxygen consumption and GDP binding in BAT. In mice only the rise in rectal and skin temperature were significantly changed by endotoxin administration.

These findings suggest that in some species BAT is involved in the production of endotoxin induced fever and increased energy expenditure.

INTRODUCTION

Fever and increased metabolic rate commonly occur after injury, irrespective of whether the injury is accidental, surgical, thermal or septic (1). The mechanisms that produce these responses are not entirely understood although inflammatory mediators and a number of stress hormones, including catecholamines, glucagon and corticosteroids are probably involved (2). In small animals endotoxin induced fever may also be due to conservation of heat by the tail (3), and to increased heat production by brown adipose tissue (BAT) (4, 5). This last suggestion has been based on two pieces of indirect evidence: (a) reports that endotoxin increases blood flow to brown adipose tissue (5, 6), and (b) the association between an increase in blood flow through BAT and non-shivering thermogenesis (activation of BAT thermogenesis) in

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animals exposed to the cold and given noradrenaline (7–10). In this study we report the results of the effects of endotoxin administration on the activity of the BAT mitochondrial proton conductance pathway (GDP binding), which plays a fundamental role in regulatory thermogenesis in small animals (11–14). The results are related to changes in whole body oxygen consumption and to changes in rectal, skin and tail temperatures.

METHODS AND MATERIALS

Animals and Experimental Procedure

Experiments were carried out on 3–4 month old male mice of the Aston variety, and male Dunn rats (approx. 300 g) of the Norwegian hooded variety. Both groups of animals were maintained on a 12 h light/12 h dark cycle (light period from 7.00 a.m.). Animals were acclimated at various temperatures for at least 3 weeks before use.

All experimental animals were given a single intraperitoneal injection (3 mg/kg) of E. coli endotoxin type 0127 B8 (butanol extracted) which was obtained from Sigma Chemical Co. Ltd., Poole, Dorset, UK. The endotoxin was dissolved in sterile saline (0.9 % w/v) at a concentration of 1 mg/ml for mice and 2 mg/ml for rats. The control animals were given saline without endotoxin.

O₂ Consumption

The effect of endotoxin on O₂ consumption was assessed in mice acclimated at thermoneutral temperature (32°C) for 3 weeks.

Preliminary studies had shown that such animals responded to endotoxin injection by producing a more pronounced and reproducible rise in rectal temperature (compared to saline controls) than animals housed at 22°C. The effect of endotoxin on oxygen consumption was also assessed in mice that were acclimated at 22°C for 3 weeks and transferred to thermoneutral temperature for 24 hr.

The rats were acclimated at 26°C ± 2°C (lower end of their thermoneutral temperature), which was shown in preliminary studies to be suitable for eliciting an endotoxin induced fever. Whole body oxygen consumption was measured continuously for six hours after endotoxin administration. The measurements were made at the thermoneutral temperature for each animal (32°C for mice and 28°C for rats) using a closed circuit oxygen consumption system (15).

Temperature Measurement

The effect of endotoxin on temperature was assessed in the following groups of animals: (a) rats acclimated at 26°C ± 2°C, (b) mice acclimated at 32°C, (c) mice acclimated at 22°C, and (d) mice acclimated at 22°C and transferred to thermoneutral temperature for 24 hr.

The measurements were made at the same temperature as that used to acclimatise the animals (except animals in group (d) which were tested at 32°C).

Rectal temperature was only measured in groups (c) (n = 6) and (d) (n = 6), while rectal, skin and tail temperatures were measured in groups (a) (n = 12) and (b) (n = 9).
Rectal, skin and tail temperatures were measured using thermocouples connected to a digital display thermometer. Skin measurements were obtained by placing the thermocouple beneath the fur on the rear flank of the animal. Tail temperatures were taken at a point 2 cm from the base of the tail. During these measurements, the thermocouples were insulated with cotton wool and a polystyrene cover. The insulation was sufficient to give a constant reading (i.e. to prevent any drift in the readings due to exchange of heat with the environment). Rectal temperatures were taken by inserting a probe to a depth of 1.5 cm for mice and 5 cm for rats.

**Brown Adipose Tissue**

In this set of experiments the effect of endotoxin on the activity of the proton conductance pathway of interscapular BAT was studied. The animals were killed by cervical dislocation 4 hours after endotoxin administration. Interscapular BAT was rapidly removed, cleaned of any adhering white adipose tissue or connective tissue, and weighed. The brown fat was then homogenized in a medium (pH 7.2) containing 250 mM sucrose, 200 μM potassium EDTA and 1 mM HEPES. Samples of the homogenate were taken for the measurement of cytochrome oxidase activity (EC 1.9.3.1) at 25°C, by a spectrophotometric method (16), and for the measurement of total tissue protein content (17).

The major portion of the BAT homogenate was used to prepare mitochondria (18), which were subsequently assayed for GDP binding (18), and the concentration of the mitochondrial uncoupling protein by a modification of a solid phase radioimmunoassay (19), as described previously (20). A rabbit anti-rat uncoupling protein serum was used in the radioimmunoassay, together with a rat protein standard.

Statistical analysis of group data was performed using the students t-test.

**RESULTS**

**O₂ Consumption**

Endotoxin produced a small non-significant rise in O₂ consumption in both the mice acclimated at 32°C (10% increase above controls) and also in those acclimated at 22°C transferred to 32°C for 24 hr before the experiment (13% increase above controls).

In the rats O₂ consumption was significantly elevated from 3 hr after endotoxin treatment (see Fig. 1). Between 3–4 hr the measurements were 19% higher (p < 0.01) than in control animals and between 4–5 hr they were 21% higher (p < 0.05). The initial measurements in both saline and endotoxin injected animals were high as a result of the injection and the change in their environment.

**Temperature Measurements**

Rats (acclimated at 26°C) and mice (acclimated at 32°C) exhibited higher rectal temperatures after endotoxin administration than after saline. A maximum difference of 1.4°C was observed in rats at 4 hr, and 0.9°C in mice at 4.5 h. These differences were
Fig. 1. Change in resting metabolic rate after saline (□) or endotoxin (■) administration in rats. *p < 0.05; **p < 0.01 compared with saline treated rats. 6 rats per group, results are mean values over one hour periods ± SEM.

Fig. 2. Effect of endotoxin on rectal, skin and tail temperature in rats (Fig. 2a) and mice (Fig. 2b). (●) Endotoxin treated animals. (○) Saline controls. Results are means ± SEM with the number of animals/group in parentheses. *p < 0.05; **p < 0.01; ***p < 0.001 significant differences between endotoxin and saline injected animals are indicated.
also reflected in skin temperatures which, at their peak, were 0.8–0.9°C greater in endotoxin injected animals than saline injected animals. However, in neither rats nor mice was there an increase in tail temperature (see Fig. 2).

In mice acclimated at 22°C, endotoxin administration caused a maximum increase in mean rectal temperature which was only 0.4°C above control animals (N.S.). The mice that were acclimated at 22°C and transferred to 32°C 24 hr before endotoxin administration responded by producing a larger rise in rectal temperature (0.7°C – p < 0.02).

**Brown Adipose Tissue Measurements**

The weight of BAT and GDP binding of BAT mitochondria in mice were not significantly affected by endotoxin administration (Table 1). In contrast injection of endotoxin to the rat caused a significant decrease in the weight of interscapular BAT (18.5%) and an increase in GDP binding (67%). These changes were not associated with significant alterations in total protein, cytochrome oxidase activity or concentration of uncoupling protein in BAT (see Table 2).

<table>
<thead>
<tr>
<th>Table 1. Effect of <em>E. coli</em> endotoxin 0127 B8 (butanol extract) on brown adipose tissue in mice</th>
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<tr>
<td>Control</td>
</tr>
<tr>
<td>Acclimatised at 32°C</td>
</tr>
<tr>
<td>BAT wt. (mg)</td>
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<tr>
<td>GDP bound (µmol/mg mito. prot.)</td>
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<tr>
<td>(n = 6 per group)</td>
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<tr>
<td>Acclimatised at 22°C/24 hr at 32°C</td>
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<tr>
<td>BAT wt. (mg)</td>
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<tr>
<td>GDP bound (µmol/mg mito. prot.)</td>
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<td>(n = 5 per group)</td>
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BAT was obtained 4 hours post endotoxin administration. Results are mean ± SEM.

**DISCUSSION**

This study demonstrates three aspects of the response of animals to endotoxin. The first relates to an apparent species difference in response to the same endotoxin. Thus, in the rat rectal temperature, metabolic rate and brown fat thermogenic indices all changed significantly after endotoxin administration, whereas in the mouse, despite similar trends to those found in the rat, significant changes in temperature could only be demonstrated with respect to temperature (and these only occurred at thermoneutral temperature). This suggests that the rat may be a better model than the mouse, for studying the hypermetabolic response to injury.
Table 2. Effect of *E. coli* endotoxin 0127 B8 (butanol extract) on brown adipose tissue in rats

<table>
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<tr>
<th></th>
<th>Control</th>
<th>Endotoxin</th>
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</thead>
<tbody>
<tr>
<td>BAT wt. (mg)</td>
<td>430 ± 21</td>
<td>355 ± 18*</td>
</tr>
<tr>
<td>GDP bound (μmol/mg mito. prot.)</td>
<td>182 ± 32</td>
<td>304 ± 28**</td>
</tr>
<tr>
<td>Cyto. oxid. activity (μmol cyto. c oxid./min.)</td>
<td>83.9 ± 8.0</td>
<td>93.0 ± 10.2</td>
</tr>
<tr>
<td>Uncoupling protein (μg/mg mito. protein)</td>
<td>24.6 ± 2.0</td>
<td>31.8 ± 3.2</td>
</tr>
<tr>
<td>Total BAT protein (mg)</td>
<td>23.3 ± 1.0</td>
<td>23.2 ± 1.2</td>
</tr>
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</table>

BAT was obtained 4 hours post endotoxin administration (n = 6 in each group).
Results are mean ± SEM.
Significant differences between endotoxin and saline injected animals are indicated: *p < 0.05, **p < 0.02.

The second point relates to tail temperature. A rise in the temperature of the blood perfusing the tail almost certainly occurred after endotoxin administration because there was a rise in rectal (core) temperature. Although this might be expected to produce a rise in tail temperature, no such rise occurred. Therefore it is reasonable to conclude that endotoxin reduced the perfusion of the tail and the proportion of total heat lost via the tail. Such conclusions are consistent with the haemodynamic changes observed in the tail of the rat after intravenous injection of a different type of endotoxin (*E. coli* 0111) (3).

Thirdly, and most importantly, the study has provided evidence that in the rat, administration of *E. coli* endotoxin activates brown adipose tissue. This is apparent from the loss of BAT weight, which is presumably associated with increased lipolysis and fat oxidation, and the increase in GDP binding in BAT mitochondria (due to unmasking of binding sites) which reflects activation of the proton conductance pathway. Cytochrome oxidase activity and the concentration of brown fat uncoupling protein, as expected, did not change during the course of this short study. The data therefore indicate that under the circumstances of study, a specialised thermogenic tissue becomes activated by *E. coli* endotoxin type 0127:B8 and contributes to the pyrexia and increased energy expenditure. The pyrexia itself may also increase energy expenditure by increasing the overall rate of metabolic processes in the various tissues of the animal.

The mice acclimatised at 32°C had a markedly depressed BAT activity, which may have restricted reactivation of the proton conductance pathway in response to endotoxin. However this should not have been a problem in the mice that were maintained at 22°C and exposed to 32°C for only 24 hr prior to endotoxin administration. Since this group of mice also showed no increase in GDP binding in its BAT mitochondria, it is suggested that mouse BAT, under the conditions of study, is not activated by *E. coli* endotoxin type 0127: B8.

Whether brown fat becomes activated after other forms of trauma and after administration of other types of endotoxins remains to be elucidated.
ACKNOWLEDGEMENTS

We wish to thank Dr P. Lunn for helpful discussions and Miss M. Howlett and Mrs A. Flack for assistance in the animal house and Miss D. Fordham for typing the manuscript.

REFERENCES