Sympathetic activation of brown-adipose-tissue thermogenesis in cachexia

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(Received 15 June 1981)

Tumour-bearing mice spontaneously lose weight 8–9 weeks after implantation of a human hypernephroma, in spite of a normal food intake. Resting oxygen consumption was up to 40% higher in these animals than in sham-operated controls, but was significantly reduced by β-adrenergic blockade with propranolol in the former group. The injection of noradrenaline caused a marked stimulation of the metabolic rate in all the animals, but the greatest response was seen in the cachectic mice. The brown-adipose-tissue mass was similar for both groups, but guanosine diphosphate binding to brown-adipose-tissue mitochondria (an index of thermogenic capacity) was significantly increased in tumour-bearing mice, and the injection of noradrenaline 1 h prior to sacrifice caused the greatest stimulation of binding in the cachectic group. These data suggest that the rapid weight loss of tumour-bearing animals may be due to a high metabolic rate which results from sympathetic stimulation of brown-adipose-tissue metabolism. The relevance of these results to cancer-induced cachexia in man is discussed.

Weight loss is commonly observed in patients with cancer and may often be a contributory cause of death (see 1–3 for reviews). This cachexia is often associated with a reduced food intake and anorexia, but a number of studies have reported increases in energy expenditure and weight loss in cancer patients consuming normal amounts of food (4–8). Several theories have been advanced to explain the high metabolic rates of some cachectic patients (see 9 for review), and these include increases in Cori-cycle activity, substrate cycles in glycolysis, ion pumping, and whole-body protein turnover, but few of these can claim any direct experimental support.

In normal animals high metabolic rates are observed in response to cold exposure or arousal from hibernation (non-shivering thermogenesis; 10,11) and elevated energy intakes (diet-induced thermogenesis; 12–14). Recent data have shown that both these phenomena involve the sympathetic nervous system and brown adipose tissue (BAT), since

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cold-adapted and hyperphagic rats exhibit high metabolic rates which are reduced by β-adrenergic blockade, enhanced thermogenic responses to noradrenaline (10-13), increased noradrenaline turnover (15), and hyperplasia and hypertrophy of BAT (13). Measurements of blood flow and oxygen extraction of BAT have revealed that this tissue can account for almost all of the enhanced thermogenic capacity of rats exhibiting non-shivering (16) and diet-induced (17) thermogenesis. Nicholls (18) has suggested that the high rates of heat production in BAT are due to the presence of a proton-conductance pathway in the inner mitochondrial membrane which dissipates the proton gradient generated by respiration and therefore allows uncoupled respiration. The activity of this pathway can be estimated from the binding of purine nucleotides to mitochondria, and such measurements have revealed a high activity of this pathway in brown fat from cold-adapted (18,19) and hyperphagic (20) rats.

In view of the established role of the sympathetic nervous system and BAT in the physiological responses to cold and diet, the present experiments were undertaken to establish whether this mechanism may also be involved in the weight loss of tumour-bearing mice. The results indicate that the cachexia of these animals is largely due to an increase in the metabolic rate which may be mediated by sympathetic stimulation of brown-fat thermogenesis.

Materials and Methods

Female mice (CBACA) obtained from OLAC (Oxford, U.K.) were maintained on Spratts Laboratory Rodent Diet at an environmental temperature of 24°C (light/dark cycle 12 h) throughout the study. When the mice were 9 weeks old the thymus was surgically removed under ether anaesthesia, and 2 weeks later these animals were injected with cytosine arabinose (200 mg/kg body wt.) and then exposed to cobalt radiation (900 rad/mouse). At 12-13 weeks of age, half the animals (T group) were implanted on the right flank with a small piece of tissue (1 mm3) containing tumour cells derived from a human hypernephroma (for further details, see 21). Control mice (C group) were sham-operated but received no tumour implantation.

Starting 6 weeks later (on day 1), the resting oxygen consumption (\(\text{VO}_2\)) was measured six times (on days 1, 5, 7, 9, 10, and 11 of the experiment), in a closed-circuit respirometer (22) for 2 h during the daytime at a temperature of 29°C. Values were taken only when the animals were resting and are corrected for body size (kg body wt.\(^{0.75}\); W.75). The resting \(\text{VO}_2\) was also measured after injections of either noradrenaline (NA; 0.25 μg/g body wt., subcutaneous) or propranolol (10 μg/g body wt., subcutaneous) in all the mice.

On day 13 of the experiment half of the C and T mice were injected with NA (as above) and the remainder with saline (0.1 ml, subcutaneous). Thirty-five minutes later all the animals were killed by cervical dislocation and the interscapular, dorsal cervical, and axillary BAT depots dissected and weighed. This tissue was then homogenized, and mitochondria were prepared (23). The binding of \(^3\)H-labelled guanosine diphosphate (GDP) to mitochondria was estimated by a method we have previously described (20). The protein content of the mitochondrial suspension was determined by the Bio-Rad method.
Fig. 1. Body weight (solid lines) and resting VO₂ (broken lines) of control (●) and tumour-bearing (○) mice. The first weighings (on d 1) were at 6 weeks after the implantation of the tumours (or, in the control mice, after the sham operations). Mean values, bar denotes SEM, n = 10. **P <0.01, ***P <0.001 in comparison with controls.

All values are presented as means ± standard error of the mean. The test of significance was Student's t-test for unmatched data, and all probabilities are two-tailed.

Results

The mean body weights of the control and tumour-bearing mice remained similar over the 7 weeks after implantation, but then the T animals began to lose weight rapidly (Fig. 1). The resting VO₂ (Fig. 1) was significantly elevated in the T mice 9 d before the onset of rapid weight loss but progressively increased over the subsequent 7 d
to reach a value 40% above that of the controls on day 11 of the experiment. This difference in resting VO\textsubscript{2} is still apparent when the values are expressed as ml/min per whole animal, since the body weights of the two groups differed by only 4% at this time.

Injections of NA (Fig. 2) produced a marked increase in the resting VO\textsubscript{2} in all the mice, but the percentage and maximum increment were significantly greater in T animals. The resting VO\textsubscript{2} after the injection of NA was similar for the control mice on day 7 (22.36 ± 1.95 ml/min/W\textsuperscript{0.75}) and day 9 (24.86 ± 1.35) but rose significantly (∗∗∗P < 0.001) in tumour-bearing mice from 29.01 ± 0.61 to 34.80 ± 0.63.

β-adrenergic blockade did not significantly alter the VO\textsubscript{2} of controls but caused a marked reduction in T mice (Fig. 2), and thus propranolol abolished 60-65% of the difference in resting VO\textsubscript{2} between the two groups.

The total mass of interscapular, dorsal cervical, and axillary BAT was reduced by 15% in T mice, but the weight of tissue/g body wt. was similar for both groups, as was the mitochondrial protein content in this tissue (Table 1). Specific binding of GDP to BAT mitochondria was doubled in the saline-injected T mice compared to controls, and in vivo administration of NA produced a further 300% increase in binding in the former group but only a 124% increase in the control mice, although a large variation in the response to NA was observed in the tumour-bearing animals.
Table 1. BAT mass, mitochondrial protein, and GDP binding in control and tumour-bearing mice

<table>
<thead>
<tr>
<th></th>
<th>No. of mice</th>
<th>Control mice</th>
<th>Tumour-bearing mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAT mass (mg)</td>
<td>10</td>
<td>125 ± 4</td>
<td>107 ± 6</td>
</tr>
<tr>
<td>BAT mass (mg/g body wt.)</td>
<td>10</td>
<td>5.93 ± 0.22</td>
<td>5.38 ± 0.24</td>
</tr>
<tr>
<td>Mitochondrial protein (mg)</td>
<td>10</td>
<td>0.93 ± 0.14</td>
<td>0.70 ± 0.09</td>
</tr>
</tbody>
</table>

Mitochondrial GDP binding (pmol/mg protein)
- Saline-injected 5 186 ± 21 373 ± 60*
- NA-injected 5 417 ± 79 1476 ± 206**

Mean values ± SEM, *P <0.05, **P <0.01 in comparison with controls.

A highly significant correlation (P <0.001; Fig. 3) was found to exist between the level of mitochondrial GDP binding in BAT from all the saline-injected mice and the resting VO₂ of these animals 1-2 d prior to sacrifice.

Discussion

Previous work on these tumour-bearing mice (21) has revealed that the cachexia which occurs 6-7 weeks after implantation can result in reductions in body weight of up to 40% in 10 d and eventually proves fatal. This emaciation cannot be attributed to reductions in food intake, since this was almost identical to that of the control animals; nor can it result from a high energy requirement of the tumour, which achieves a mass equivalent to only 5% of the body weight of the host at the time of weight loss.

In the present study cancerous animals showed elevated metabolic rates which increased rapidly over 2-3 d preceding the onset of weight loss. These results contrast with the marked reductions in metabolic rate seen in animals which have lost weight due to food restriction (24). The differences in resting VO₂ between the cachectic and the normal mice were apparently not due to alterations in physical activity, since values were taken only when the animals were resting and casual observations of behaviour indicated a greater activity in the control mice. The marked effects of propranolol on tumour-bearing animals implies that the high levels of oxygen consumption resulted from increased sympathetic activity. The dose of propranolol used did not completely abolish the differences in resting VO₂ between the two groups, but we have noted (N. J. Rothwell & M. J. Stock, unpublished
data) that a higher level (20 μg/g) is often required for complete inhibition of non-shivering and diet-induced thermogenesis in the rat. It is therefore possible that increasing the amount of propranolol might have caused a further reduction in resting VO\textsubscript{2} in T mice; but this was avoided because of the prolonged duration of β-blockade which occurs after injecting high doses of propranolol, since this may have affected the course of weight loss.

The enhanced thermogenic response to NA in tumour-bearing mice is also indicative of an increased sympathetic activity, since chronic elevations in catecholamine levels are known to potentiate the effects of noradrenaline on resting VO\textsubscript{2} (25). It is interesting that the NA-stimulated resting VO\textsubscript{2} in cancerous animals was greatest on day 9, shortly before the onset of weight loss, when the resting VO\textsubscript{2} had also increased.
Studies on the rat and the mouse have shown that the main effector tissue for the thermogenic actions of NA is BAT, and the responses to propranolol and NA in the mice are remarkably similar to those reported in cold-adapted and hyperphagic animals (10,12), in which brown-fat activity is very high. It is therefore likely that changes in BAT thermogenesis may also be involved in the high metabolic rates of tumour-bearing mice, and the data presented in Table 1 support this assumption. The small reduction in the brown-fat mass of implanted mice is probably due to the mobilization and oxidation of triglyceride which usually occurs in active brown fat, particularly when energy expenditure is greater than intake. However, the mass of BAT as a proportion of body weight was unaltered. Earlier studies involving electron microscopy of BAT from these mice (S. Wilson, unpublished data) indicated structural alterations in the mitochondria from cachectic mice resembling those seen in tissue taken from cold-adapted animals, which are not present in normal mice that have been starved to cause the same degree of weight loss.

The two-fold increase in mitochondrial GDP binding in BAT from the T group (Table 1) is also reminiscent of data from cold-adapted (19) and hyperphagic (20) animals and suggests an increased activity of the proton-conductance pathway in mice implanted with the tumour. Nicholls (18) has proposed that a high activity of this pathway, which results in uncoupled respiration, is responsible for the elevated metabolic rates exhibited by cold-adapted animals, but at present it is impossible to quantify the contribution of this mechanism to in vivo heat production. The highly significant correlation between in vitro GDP binding in BAT and in vivo resting VO$_2$ of control and cachectic mice indicates a strong relationship between these parameters, although this may not be a causal link.

Overall, the results of this study suggest that the weight loss of tumour-bearing mice is due to a high metabolic rate which may result from sympathetic activation of BAT, though the mechanisms by which the tumour induces these changes in host metabolism are as yet unknown.

The alterations of resting VO$_2$ in the cancerous mice (40% increase) are remarkably similar to those reported by Warnold et al. (8) in human cancer patients (39% increase), but the possibility that the same mechanism could operate in cachectic man has not yet been investigated. In normal subjects there is some data to suggest that the sympathetic nervous system may be involved in the thermogenic responses to cold (26) and diet (27), but although adult man does possess brown fat (28) the activity of this tissue has not been studied. Wilmore et al. (29) have presented evidence to suggest that the hypermetabolism of patients who have sustained serious burns may be related to an increase in catecholamine levels, and the data presented here suggest that this mechanism could also be important in cancer-induced cachexia.

References


