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THE ROLE OF BROWN ADIPOSE TISSUE IN THE CALORIGENIC EFFECT OF ADRENALINE AND NORADRENALINE IN COLD-ACCLIMATED RATS

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SUMMARY

1. Removal of the interscapular brown adipose tissue of the cold-acclimated rat has no immediate effect on the calorigenic response of the rat to adrenaline or noradrenaline.

2. There is a progressive loss of the enhanced response to adrenaline and to noradrenaline during the 4 days following removal of the interscapular brown adipose tissue from cold-acclimated rats. There is no loss of response in sham-operated cold-acclimated rats.

3. Removal of the interscapular brown adipose tissue from rats living at room temperature has no effect on the calorigenic response to adrenaline or noradrenaline, neither immediately afterwards nor 2–4 days later.

4. It is concluded that interscapular brown adipose tissue is not the major site of oxygen consumption in the enhanced calorigenic response to adrenaline or noradrenaline in cold-acclimated rats. However, it does play an important role in this enhanced metabolic response, probably as an endocrine gland whose secretory product modifies the ability of other tissues to respond calorigenically to catecholamines.

INTRODUCTION

Although it is well established that the calorigenic effect of *noradrenaline* is greatly enhanced in cold-acclimated rats (see Himms-Hagen, 1967, for review), an enhancement believed to be the major basis for nonshivering thermogenesis in these animals, the only reports available about the effect of *adrenaline* (Hsieh & Carlson, 1957; Ring, 1942; Swanson, 1957) suggest little or no change in its effect in cold-acclimated rats. This is probably

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because the subcutaneous (Ring, 1942) or intramuscular (Hsieh & Carlson, 1957; Swanson, 1957) routes of administration used are not suitable for demonstrating such an effect. The experiments to be described in this paper were initiated with the aim of comparing the calorigenic effect of intravenously infused adrenaline with the calorigenic effect of intravenously infused noradrenaline in cold-acclimated rats in order to have a better background of information for considering the mechanism of this process.

Since preliminary experiments indicated that the calorigenic effect of intravenously infused adrenaline was indeed enhanced in cold-acclimated rats to about the same extent as was the calorigenic effect of noradrenaline, a further study was made in which the effect of removal of the interscapular brown adipose tissue on the calorigenic action of these two catecholamines was studied. Hull & Segall (1965) had reported that removal of the interscapular brown adipose tissue in new-born rabbits practically abolished the calorigenic effect of noradrenaline and Leduc & Rivest (1969) had reported that its removal could reduce the calorigenic effect of noradrenaline in cold-acclimated rats. In the course of these experiments, evidence (Himms-Hagen, 1969) was obtained which suggested a possible endocrine function of the interscapular brown adipose tissue and this is the principal subject of this paper.

METHODS

Male white rats (Sprague–Dawley) were purchased from the Holtzman Company at a weight of 150 g. They were kept in large cages at 25–28° C (this will be referred to as room temperature) until they reached 200–220 g at which time they were placed in individual wire mesh cages at 4° C (cold-acclimated rats) or at room temperature (warm-acclimated rats). Fluorescent lighting was on for 12 hr per day (6 a.m. to 6 p.m.) and the rats had free access to food and water. The rats remained under these conditions for up to 11 weeks. The weights of the rats at the end of the acclimation period and the length of this period are given in the legends to the Figures. Rats were brought to the laboratory (25–28° C) for a specified time before the experimental procedure; this time is also indicated in the legends to the Figures.

For the measurement of oxygen uptake rats were lightly anaesthetized with sodium pentobarbitone (2·8 mg/100 cm^2 body surface, administered I.P.) and a polyethylene cannula placed in a tail vein. Sodium chloride solution (0·9 g/100 ml., referred to as saline) was infused at a rate of 0·021 ml./min. For a period of 30 min the saline infusion contained adrenaline or noradrenaline which was infused at a rate of 0·5 μg/100 cm^2 body surface.min. This dose produces a maximum calorigenic effect; larger doses do not cause any larger effect and frequently kill the animal. The rat was placed in a metabolic chamber in which the oxygen uptake was measured with an open-circuit system (with a flow rate of 477 ml. air/100 cm^2 body surface. min) and a Westinghouse oxygen analyser.

The interscapular brown adipose tissue (comprising approximately 40 % of the total rather diffuse brown adipose tissue of the body) was removed under ether anaesthesia. In sham-operated animals a similar skin incision was made under ether anaesthesia but the interscapular brown adipose tissue was not disturbed. In some

experiments the rats were kept at room temperature for up to 4 days following the operation. In those experiments in which the calorigenic response to catecholamine was studied immediately after the operation a slightly different procedure was followed. The rats were injected with pentobarbitone (80 % of the usual dose), immediately anaesthetized with ether, and then the operation was performed; the administration of ether was stopped during the operation. Rats did not regain consciousness but remained lightly aneasthetized by the pentobarbitone. Infusion of catecholamine was not started until the resting oxygen uptake was steady and in the normal range; this was usually within 1 hr after the operation.

Adrenaline and noradrenaline (both bitartrates) were dissolved in 0·01 N hydrochloric acid at a concentration of 10 mg base/ml. These solutions were diluted in saline (approximately 0·5–1 in 100) immediately before use.

RESULTS

Calorigenic response to adrenaline. The time course of the oxygen uptake during infusion of adrenaline is shown in Fig. 1 to illustrate the method of expression of results. The oxygen uptake rises rapidly and reaches a new, fairly steady level which is higher in the cold-acclimated rat than in the warm-acclimated rat; the resting oxygen uptakes are similar in all rats and are within the range 1·40–1·90 ml./100 cm^2. min. The measurement of response used here is the area under the curve during the 30 min. of infusion and above the dashed line representing the continuation of the resting level of the oxygen uptake; it is expressed as ml. oxygen used/100 cm^2 body surface in 30 min.

Development of enhanced calorigenic response during acclimation to cold. The metabolic response to adrenaline increases rapidly during the first three weeks of acclimation to cold and then remains at a high level (Fig. 2). This is very similar to the development of enhanced metabolic response to noradrenaline during acclimation to cold (Depocas, 1960). The response varies little whether the rats are infused within 1 hr of removal from the cold or if they are kept at room temperature for 24 hr before the infusion (Fig. 2).

Effect of removal of the interscapular brown adipose tissue. In preliminary experiments the interscapular brown adipose tissue was removed from rats immediately after they were removed from the cold; the rats were allowed to recover at room temperature for 24 hr before the infusion. This procedure reduced the enhanced response to adrenaline by 50 % in rats that had lived in the cold for 4–6 weeks and almost completely in rats that had been in the cold for only 1–2 weeks (Fig. 3).

Because the small amount of interscapular brown adipose tissue removed was unlikely to have been the actual site of oxygen consumption in the enhanced response to adrenaline in the intact animal (see Discussion) the working hypothesis was formulated that the tissue was playing another role, possibly that of an endocrine organ, in the enhancement of

the response to adrenaline in cold-acclimated rats. If this were so, then the 24 hr waiting time used in the experiments shown in Fig. 3 might not have been the most suitable, and further preliminary experiments were performed to follow the time course of disappearance of response (Fig. 4). The response is lost progressively during the 4 days following the operation; immediately after the operation there is no effect of removal of the inter-

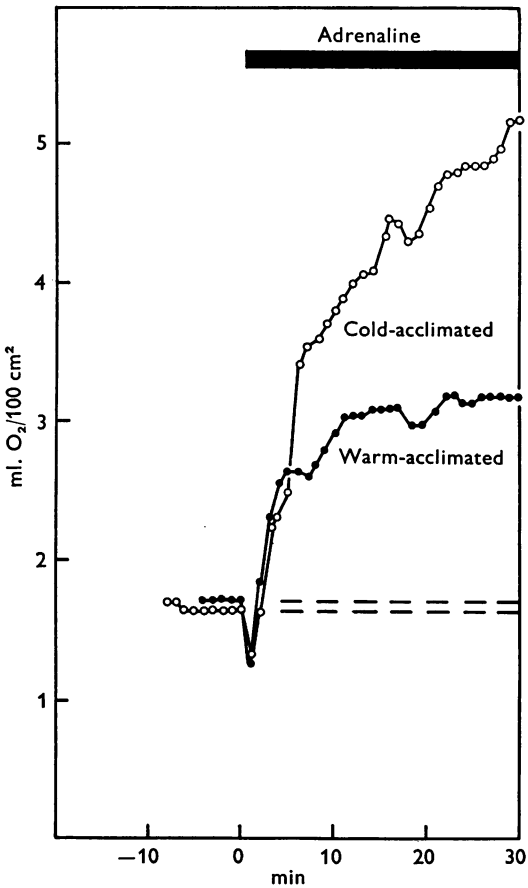

Fig. 1. Effect of intravenous infusion of adrenaline on oxygen uptake of a warm- and a cold-acclimated rat. Adrenaline, 0·5 μg/100 cm^2 body surface. min was infused via a tail vein from 0–30 min. The warm-acclimated rat weighed 415 g, the cold-acclimated rat weighed 331 g; they had lived at room temperature (25–28° C) or at 4° C for 9 weeks and the cold-acclimated rat received the infusion within 1 hr of removal from the cold. The metabolic response shown in all subsequent Figures is the area of the curve of oxygen uptake above the dashed line at the resting level of oxygen uptake. In the two examples shown here the metabolic response was 30·6 ml. oxygen/100 cm^2 in 30 min for the warm-acclimated rat and 62·8 ml. oxygen/100 cm^2 in 30 min for the cold-acclimated rat.

scapular brown adipose tissue on the calorigenic response to adrenaline (Fig. 4). This result clearly shows that the interscapular brown adipose tissue cannot itself be an important site of oxygen uptake in the enhanced calorigenic response to adrenaline in cold-acclimated rats.

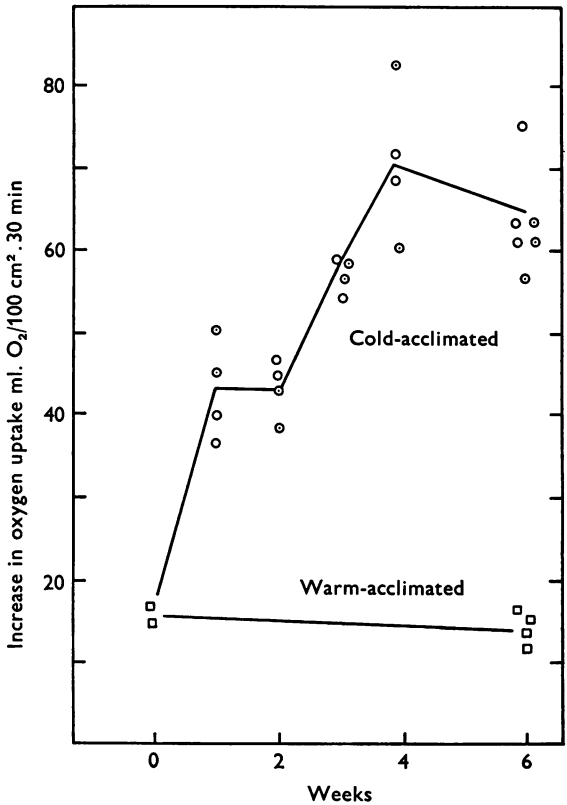

Fig. 2. Development of an enhanced calorigenic response to adrenaline during acclimation to cold. Warm-acclimated rats (□) or cold-acclimated rats that had been in the warm for 1 hr (⊙) or for 24 hr (○) received an intravenous infusion of adrenaline. The calorigenic response is plotted against the duration of acclimation. The mean weight of the rats at zero time was 208 g; at 6 weeks the cold-acclimated rats weighed 306 g and the warm-acclimated rats weighed 369 g.

Further experiments were designed to establish firmly this progressive loss of response to adrenaline after removal of the interscapular brown adipose tissue and to find out if the enhanced response to noradrenaline were also lost progressively. Figure 5 illustrates the decline in the response to adrenaline; at zero time no change is observed, whereas at 2–3 days the response is almost down to the level in the warm-acclimated rats. Removal

of this tissue does not influence the calorigenic response to adrenaline in the warm-acclimated rats. The experiments illustrated in Fig. 6 show that the enhanced calorigenic response to noradrenaline in cold-acclimated rats is similarly unaffected immediately after removal of the interscapular brown adipose tissue but is very much reduced 4 days later.

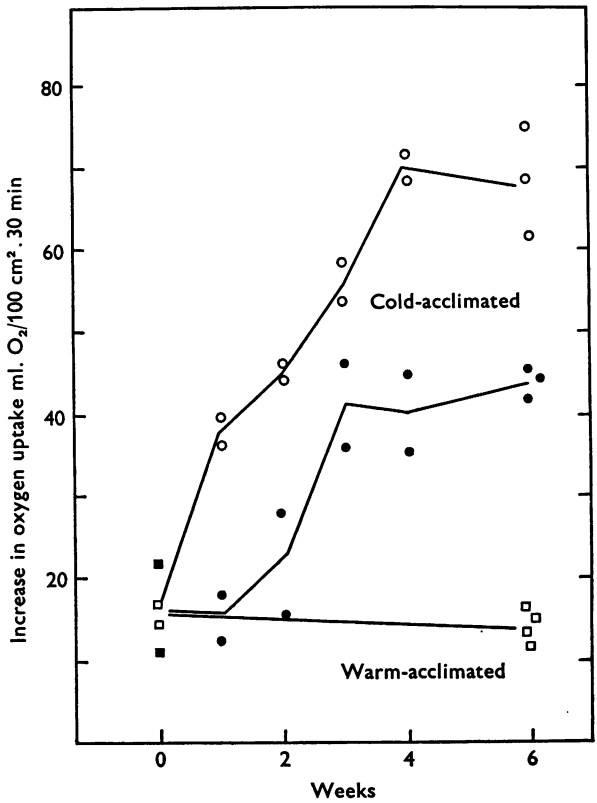

Fig. 3. Effect of removal of the interscapular brown adipose tissue 24 hr previously on the calorigenic response to adrenaline in cold-acclimated rats. Rats without their interscapular brown adipose tissue are represented by filled symbols (●, cold-acclimated rats; ■ warm-acclimated rats at zero time). Unoperated rats (○, cold-acclimated; □, warm-acclimated) are also shown for comparison. Mean weights of the operated rats were 213 g at zero time and 308 g at 6 weeks. Removal of the interscapular brown adipose tissue did not alter the resting level of oxygen uptake.

DISCUSSION

The first conclu sion to be drawn from these results is that the interscapular brown adipose tissue is not itself a major site of oxygen uptake responsible for the enhanced calorigenic response to adrenaline or to nor-

adrenaline in cold-acclimated rats; this conclusion is based on the finding of no change in this enhanced response shortly after removal of the interscapular brown adipose tissue. Obviously the extra oxygen must be used by other tissues. Other evidence supports this same conclusion. The magnitude of the decrease in enhanced calorigenic response to adrenaline

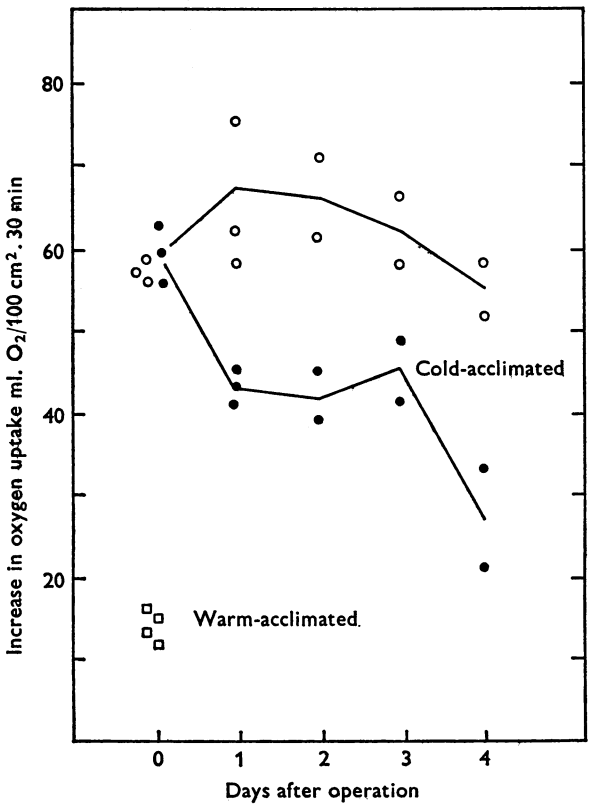

Fig. 4. Time course of the loss of the enhanced calorigenic response to adrenaline after removal of the interscapular brown adipose tissue from cold-acclimated rats. The cold-acclimated rats had lived at 4° C for 6–8 weeks; their mean weight at the time they were put into the cold was 216 g and after 6–8 weeks in the cold their mean weight was 315 g. Rats were either sham-operated (○) or had their interscapular brown adipose tissue removed (●) immediately after coming from the cold to room temperature. Adrenaline was infused within 1 hr of the operation (time zero on the graph) or up to 4 days later.

or to noradrenaline seen 4 days after removal of the interscapular brown adipose tissue is too large to be accounted for by removal of the oxygen-consuming tissue itself. The difference shown in Figs. 4 and 6 of approximately 1 ml. oxygen/100 cm² min. would require an oxygen uptake of the

tissue removed (0·17 g/100 cm²) of approximately 6 ml./g tissue.min. This is at least ten times higher than the highest ever reported for brown adipose tissue *in vivo* in the presence of noradrenaline (Heim & Hull, 1966). Moreover, the blood flow necessary to permit such an oxygen uptake (about 70 ml./g.min) would have to be at least twenty times higher than that reported for brown adipose tissue of new-born rabbits *in vivo* in the presence of noradrenaline (Heim & Hull, 1966) or of cold-acclimated rats in the cold (Jansky & Hart, 1968); it would indeed have to be almost half

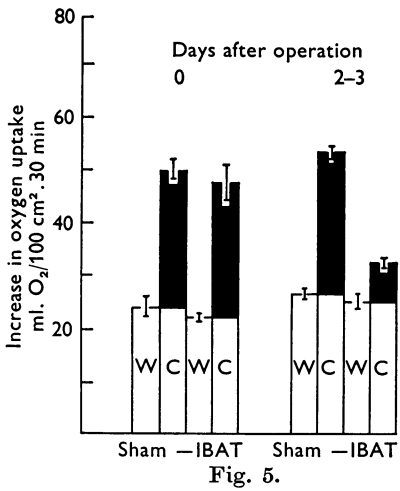

Fig. 5.

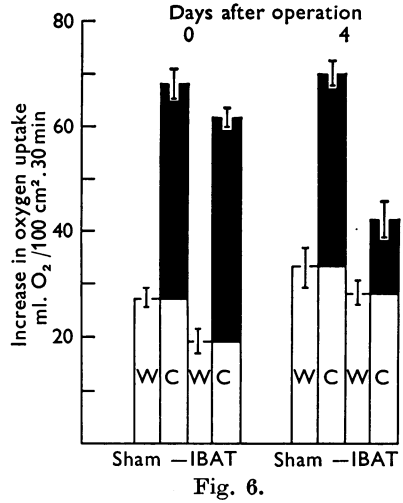

Fig. 6.

Fig. 5. Delayed effect of removal of the interscapular brown adipose tissue on the enhanced calorigenic response to adrenaline in cold-acclimated rats. Each bar represents the mean of values from ten rats with the S.E. indicated by the vertical line. The mean weight of the cold-acclimated rats at the time they were removed from the cold was 265 g; they had lived in the cold for only 1–2 weeks. The mean weights of the warm-acclimated rats at this time was 309 g. Warm-acclimated rats are indicated by W; cold-acclimated rats are indicated by C. The portion of the bars for cold-acclimated rats that is coloured black is the difference in response between cold-acclimated rats and the corresponding warm-acclimated rats and represents the enhancement of the calorigenic response to adrenaline caused by acclimation to cold. Values obtained immediately after the operation are shown on the left of the diagram (day 0); those obtained 2–3 days later are shown on the right. Sham-operated controls are labelled Sham; rats with their interscapular brown adipose tissue removed are labelled —IBAT.

Fig. 6. Delayed effect of removal of the interscapular brown adipose tissue on the enhanced calorigenic response to noradrenaline in cold-acclimated rats. Each bar represents the mean of values from six rats with the S.E. indicated by the vertical line. The mean weight of the cold-acclimated rats at the time they were removed from the cold was 332 g; they had lived in the cold for 6–11 weeks. The mean weight of the warm-acclimated rats at this time was 432 g. Other symbols are the same as in Fig. 5.

the cardiac output of the cold-acclimated rat infused with noradrenaline (Evonuk & Hannon, 1963) or exposed to cold (Jansky & Hart, 1968). The participation of tissues other than the interscapular brown adipose tissue in the enhanced calorigenic response to noradrenaline in cold-acclimated rats has been directly demonstrated in the case of skeletal muscle (Jansky & Hart, 1963; Jansky, 1966) and can be deduced from the correlation between organ cytochrome oxidase content and maximum oxygen consumption in cold-acclimated rats (Jansky, 1963). Moreover, calculations based on blood flow suggest a maximum possible contribution by brown adipose tissue of 6–8% of the total oxygen consumption of the cold-acclimated rat exposed to cold (Jansky & Hart, 1968; Imai, Horwitz & Smith, 1968).

In animals exposed to cold the interscapular brown adipose tissue may indeed have an important local function. Smith has emphasized the importance of the location and vascular connexions of the interscapular brown adipose tissue in providing a local heat source for thoracic organs and the spinal cord (Smith & Roberts, 1964; Smith & Horwitz, 1969). That this local production of heat may be important in the suppression of shivering is suggested by the close association, in an animal capable of non-shivering thermogenesis, the new-born guinea-pig, of the brown adipose tissue and the thermal sensory receptors of the spinal cord which have been postulated to be responsible for induction of shivering (Brück & Wünnenberg, 1966; Wünnenberg & Brück, 1968*a*, *b*).

The second conclusion to be drawn from the evidence presented here is that the interscapular brown adipose tissue of cold-acclimated rats must have some function other than heat production essential for the enhanced calorigenic response to adrenaline and noradrenaline. The working hypothesis presented here is that the interscapular brown adipose tissue secretes a 'cold-acclimation factor'. It seems highly unlikely that this factor could be a substrate needed to support oxidative processes in other tissues; the long time needed for the response to disappear and the plentiful supply of substrates from other sources argue against this possibility. A more likely nature of the factor is that of a hormone which modifies the function of other organs in such a way that their ability to respond calorigenically to adrenaline and noradrenaline is increased. An endocrine function for brown adipose tissue has of course been postulated in various connexions for a number of years (see Johansson, 1959; Kayser, 1961), particularly in relation to induction of hibernation. An endocrine-like appearance of interscapular brown adipose tissue of cold-acclimated rats has also been reported (see Smalley & Dryer, 1967). The hormone proposed here is not one that would itself alone alter metabolic rate; the resting oxygen uptake

of rats without their interscapular brown adipose tissue for 4 days is the same as that of intact rats.

The interscapular brown adipose tissue, therefore, may be postulated to have two related functions in the development of nonshivering thermogenesis during acclimation to cold. First, it may progressively suppress shivering by virtue of its special location and increased heat-producing capacity (see Smith & Horwitz, 1969). Secondly, it could permit, by virtue of a possible endocrine function, the development of the capacity of other body organs for nonshivering thermogenesis, i.e. the ability to respond calorigenically to noradrenaline and adrenaline. This dual function of the interscapular brown adipose tissue may play a role in the coincident decrease in shivering and increase in nonshivering thermogenesis which occur in rats during the first 4 weeks of acclimation to cold (Hart, Heroux & Depocas, 1956; Depocas, 1960).

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