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DEFECTIVE COLD-INDUCED STIMULATION OF THYROXINE 5'-DEIODINASE IN BROWN ADIPOSE TISSUE OF THE GENETICALLY OBESE (ob/ob) MOUSE

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SUMMARY: Exposure of a normal lean mouse to cold (14°C) for 12 h increases the activity of thyroxine 5'-deiodinase in brown adipose tissue 26-fold. In contrast, exposure of the genetically obese, ob/ob, mouse to cold results in little more than a doubling of thyroxine 5'-deiodinase activity. The physiological significance of endogenous 3,5,3'-triiodothyronine production in brown adipose tissue is not understood. However, it seems likely that defective cold-induced stimulation of the 5'-deiodinase in brown adipose tissue of the ob/ob mouse might cause a relatively hypothyroid state of the tissue. Thyroid hormone is known to be required for a normal thermogenic response of brown adipose tissue to noradrenaline. It is suggested that the defect in the response of the 5'-deiodinase in the ob/ob mouse could contribute to the defective thermogenic response of brown adipose tissue to cold-exposure and to noradrenaline.

INTRODUCTION: The genetically obese (ob/ob) mouse that is maintained at a temperature close to thermoneutrality has brown adipose tissue that does not become thermogenically active in the normal way when the mouse is exposed to cold (1-3). A refractoriness of the tissue to noradrenaline (4) is associated with a deficit in energy expenditure and a high metabolic efficiency that contribute to the obesity (5,6). In addition, the ob/ob mouse maintains itself in a state of torpor (regulated low body temperature) (7,8) that also contributes to its high metabolic efficiency.

The low resting metabolic rate of the ob/ob mouse (9) is not due to a hypothyroid state since serum concentrations of thyroid hormones are normal or even elevated in the adult animal (10, 11.) Yet treatment of the ob/ob mouse with thyroxine (T_4) results in an exaggerated thermogenic response (12,13) and an improvement in the

ABBREVIATIONS: HEPES, N-2-hydroxylethylpiperazine-n'-2-ethanesulfonic acid; SEM, standard error of the mean; PTU, 6-propyl-2-thiouracil; T_4, thyroxine or 3,5,3',5'-tetraiodothyronine; T_3, 3,5,3'-triiodothyronine.

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response of brown adipose tissue to cold-exposure (13) without any change in sympathetic nervous system activity in the tissue (14). Thyroid hormone is known to be essential for the thermogenic response of brown adipose tissue to cold (15). Yet this organ is not a site of thyroid-induced thermogenesis (16,17). Rather, thyroid hormone appears to be required for several of the intracellular events which occur during the thermogenic response of brown adipose tissue to noradrenaline (18); these include the activation of adenylate cyclase via an action on ß-adrenergic receptors, the cyclic AMP-induced activation of hormone-sensitive lipase, and the fatty acid-induced activation of mitochondrial respiration (18).

Recent evidence has demonstrated the presence in rats of a T_4 5'-deiodinase in brown adipose tissue that differs from the related enzyme in liver and kidney (19,20). The activity of this enzyme is markedly increased by acute cold-exposure, an effect mediated by an action of noradrenaline on α_1-adrenergic receptors (19). Thus, it seems that brown adipose tissue may not depend upon serum T_3 but rather upon generation of its own T_3 from serum T_4 when required for stimulated thermogenesis.

The objective of the experiments was to find out whether brown adipose tissue of the ob/ob mouse was able to respond to acute cold-exposure by an increase in T_4 5'-deiodinase activity and thus able to generate the T_3 needed by the tissue for a normal thermogenic response to noradrenaline.

METHODS: Female C57BL/6J mice, obese (ob/ob) and lean (+/+ or +/ob) littermates, were obtained at an age of 4-5 weeks from Jackson Laboratories, Bar Harbor, ME. They were housed in pairs (one lean, one obese) in plastic cages at 28°C in a 12 h light cycle (lights on at 0600 h) with free access to food (Purina chow 5012) and water. Pairs of mice were exposed at 4 months of age to 14°C or to 28°C for 12 h, starting at 2130 h.

Rectal temperatures were measured, then the mice were killed by cervical dislocation and blood collected. Interscapular and subscapular brown adipose tissue was removed and placed in ice-cold buffered medium (0.25 M sucrose, 1.0 mM HEPES, 0.2 mM EDTA, potassium salt, at pH 7.2). White gonadal adipose tissue was removed and weighed. Brown adipose tissue was cleaned, weighed, and homogenized in an all-glass homogenizer. Samples of homogenates were frozen immediately in liquid nitrogen and stored at -80°C. Enzyme assays were performed within one week. The enzyme was shown to be stable for at least 4 months under these conditions. Protein content was estimated by a modified Lowry method (21). T_4 5'-deiodinase was

measured (22) using [^{125}I]thyroxine as substrate and incubating for 30 min at 37°C under nitrogen. [3',5'-^{125}I]Thyroxine was obtained from Amersham with a specific activity greater than 1200 μCi/μg. It was purified by paper electrophoresis (23) immediately before use to give a free iodine content of less than 1%. A methanol: ammonia (99:1) mixture was used to extract T_4 after the electrophoresis (24). The incubation contained approximately 50,000 cpm per assay tube, 2.57 nM thyroxine, 10 mM dithiothreitol, 1 mM PTU, and 60 μg homogenate protein. Preliminary studies showed the rate of [^{125}I] liberation to be linearly related to protein content at this concentration of protein. Maximum activity was at pH 7.0 and was not inhibited by PTU up to a concentration of 1 mM. Total serum T_4 and T_3 were measured by radioimmunoassay using Amerlex kits (Amersham). Values are reported as means ± SEM. An unpaired Student's t-test was use to assess significance of differences between means. A P value of 0.05 or less was taken as indicating a statistically significant difference.

RESULTS: Specific and total activity of T_4 5'-deiodinase were slightly higher in brown adipose tissue of ob/ob mice adapted to 28°C than in lean mice (Table 1, Fig. 1). However, after 12 h of cold-exposure, the specific activity increased 26-fold in the lean mice whereas it little more than doubled in the obese mice (Table 1, Fig. 1).

Obese mice of this age weigh considerably more than their lean littermates, have more white adipose tissue and a lower rectal temperature (Table 1). The level of T_3 in their blood is normal and that of T_4 slightly elevated (Table 1). Exposure to cold induced a slight hypothermia in the lean mice and a marked hypothermia in the ob/ob mice. No change in concentrations of T_3 or T_4 occurred in lean mice; a small decrease in T_3 level occurred in cold-exposed ob/ob mice (Table 1).

DISCUSSION: There is a considerable reduction in cold-induced stimulation of T_4 5'-deiodinase activity in brown adipose tissue of ob/ob mice. The failure of this response resembles the defective cold-induced stimulation of brown adipose tissue mitochondrial GDP binding in this animal (1,2), indicative of reduced thermogenic activation of the tissue. We know that sympathetic nervous system activity is increased in brown adipose tissue of the cold-exposed ob/ob mouse (25,26). Hence, it would appear that the action of noradrenaline to increase T_4 5'-deiodinase activity in brown adipose tissue may be attenuated in the ob/ob mouse. The actions of

TABLE 1: Effect of cold-exposure (12 h at 14°C) on lean and ob/ob mice

	LEAN		OBESE	
Exposure	28°C	14°C	28°C	14°C
Number of animals	(6)	(6)	(6)	(6)
Body weight, g	22.4 ± 0.8	21.3 ± 0.7	54.1 * ±1.8	55.2 * ± 2.4
Rectal temperature, °C	37.6 ± 0.2	36.3† ± 0.5	35.8 * ±0.7	21.8 †* ± 0.9
Gonadal white adipose tissue, g	0.45 ± 0.06	0.39 ± 0.06	3.80* ±0.20	4.00* ± 0.30
Brown adipose tissue				
Wet weight, g	0.14 ± 0.01	0.12 ± 0.01	0.89* ±0.07	1.20* ± 0.20
Protein, mg	7.4 ± 0.5	7.7 ± 0.9	14.0 * ±1.4	14.6 * ± 1.6
T_4 5'-deiodinase total pmol.h^{-1}	0.46 ± 0.07	11.89† ± 1.68	1.64 * ±0.24	4.50 †* ± 1.14
Serum				
T_4, µg/dl	5.6 ± 0.3	5.2 ± 0.3	7.3 * ±0.3	6.3 ± 0.4
T_3, ng/dl	78.2 ± 11.0	93.2 ± 11.4	104.2 ±8.7	81.6 † ± 9.8
T_3/T_4, %	1.42 ± 0.14	1.80 ± 0.14	1.44 ±0.07	1.34 ± 0.15

* Significant effect of obesity ($P < 0.05$), comparing mice at the same temperature

† Significant effect of cold ($P < 0.05$), comparing the same type of mouse

noradrenaline to stimulate adenylate cyclase (27,28) and to promote thermogenesis (4) are both reduced in ob/ob mice of this age and housed under these conditions.

The action of noradrenaline to increase T_4 5'-deiodinase activity in brown adipose tissue is believed to be mediated by α_1-adrenergic receptors in rats (19). In contrast, the thermogenic action of noradrenaline on brown adipose tissue involves primarily ß-adrenergic receptors (29), although a contributory role of α_1-adrenergic receptors

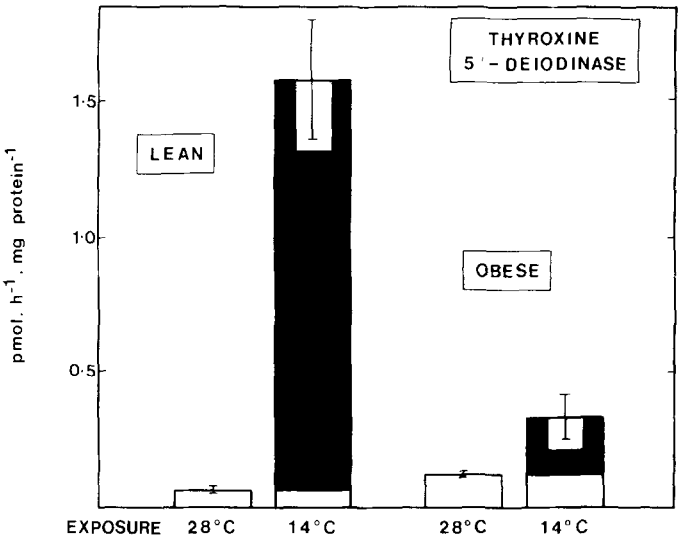

FIG. 1: Effect of cold-exposure (12 h at 14°C) on thyroxine 5'-deiodinase specific activity in brown adipose tissue homogenates of lean and ob/ob mice. Other information about these mice is in Table 1.

has been observed (30). Thyroid hormone is essential for the thermogenic action of noradrenaline on brown adipose tissue (15,18). A refractoriness of brown adipose tissue of the ob/ob mouse to the normal level of T_3 in the blood has previously been postulated to be the explanation for its poor thermogenic response to noradrenaline (31). This postulate should now be modified to indicate that the reduced endogenous production of T_3 from T_4 when the sympathetic nerve supply to brown adipose tissue is stimulated, as during acute exposure to cold, results in a failure of this tissue in the ob/ob mouse to respond adequately to the action of noradrenaline on ß-adrenergic receptors by an increase in thermogenesis.

The reason for the higher basal activity of T_4 5'-deiodinase in brown adipose tissue of ob/ob mice living at a temperature that is close to thermoneutrality is not clear. It may represent a long-term adaptation to the hyperphagic and hyperinsulinemic state of these animals.

Thus, the ob/ob mouse has a defective response to cold-induced stimulation of T_4 5'-deiodinase activity in its brown adipose tissue. Since the functional significance of endogenous T_3-neogenesis in brown adipose tissue is not understood, the consequences of a reduction in this process remain to be determined. We suggest that the defect would contribute to the defective thermogenic response of the tissue to noradrenaline.

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