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ALCOHOL DEHYDROGENASE ACTIVITY IN MOUSE BROWN ADIPOSE TISSUE*

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Abstract : The present work provides evidence for the occurence of the enzyme alcohol dehydrogenase (ADH) in very minute concentration in mice brown adipose tissue (BAT). Mice consuming 10% ethanol for 10 days showed significantly lowered enzyme activity in brown fat while liver ADH activity was increased but not significantly. Measurements of basal and norepinephrine stimulated oxygen consumption of isolated brown adipocytes indicated that the presence of ADH in BAT of mice is unlikely to play any role in ethanol oxidation.

Key words : alcohol dehydrogenase brown adipose tissue ethanol

INTRODUCTION

It is well known that the enzyme alcohol dehydrogenase (ADH) is present abundantly in liver (1). Other tissues such as the heart, lungs, kidney, retina and gastrointestinal tract of several species and almost every organ of rat possess ADH activity (2, 3). Although, the role for ADH in the degradation of ethanol in liver is well established (1, 4), the importance of ADH atleast, in some other organs is not clear (2). A previous report recognises the presence of ADH in rat brown adipose tissue (ADH) using histochemical and biochemical techniques (5). A possible role for this enzyme in rat BAT in ethanol degradation has also been suggested (6). BAT is a unique heat producing organ located in several regions of the body particularly containing a large mass in the inter-scapular space. The thermogenic function of BAT is by way of uncoupling oxidative phosphorylation in the mitochondria (7, 8) and is the key instrument in thermoregulation and body weight control (9). BAT thermogenic capacity is influenced by a number of factors such as diet, exercise, pregnancy and so on (10). Ethanol is a energy rich compound and contributes considerably to the daily energy intakes of a large number of population all over the world (11, 12). Our unpublished data showed that ethanol consumption in mice over a five week period did not alter the BAT thermogenic capacity and was comparable to water drinking controls (13). This experiment was thus designed to investigate into ADH activity in mice BAT and its possible role in the oxidation of ethanol as species differences in BAT functions are recognised to some extent (9, 10).

METHODS

CD_1 strain male albino mice weighing 25-30 g were obtained from the Animal Resource

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Center and were allowed to acclimatize to the laboratory conditions for about one week. They were housed at 22-23°C in individual wire meshed bottom cages and given Purina rodent chow 5001 *ad libitum* throughout the period of experiment. Total energy content of Chow was 17.8 kJ/g and consisted of 23.4% protein and 4.5% fat. A group of 6 mice were given *ad libitum* 10% ethanol solution in water (vol/vol) only for 10 days while another group of 6 mice were provided tap water freely for the same period of time served as controls. The calorie content of ethanol was derived as explained by Di Battista (14). Food and fluid intakes were measured twice weekly. Corrections for spillage and water loss were made. On day 11, all mice were sacrificed by cervical dislocation and interscapular and subscapular BAT was removed from each mice, cleared of other adhering tissues, blotted and weighed. A small piece of liver was also taken, washed in buffer, blotted and weighed. Immediately, the tissue was homogenised in 0.1 M glycine sodium hydroxide buffer (pH 9.6) and centrifuged at 10,000 rpm for 5 min. The supernatant was used for the estimation of ADH activity based on the measurement of NADH produced in the presence of excess alcohol according to the method of Bonnichsen and Brink (15). Optical density readings were taken from a flourimeter setting the excitation wavelength at 340 nm and emission wavelength at 475 nm.

Measurement of oxygen consumption of isolated brown adipocytes:

Isolation of brown adipocytes is described elsewhere (16). In brief, BAT pad was isolated and cut into 1 mm squares with a tissue chopper. The tissue was placed in a vial containing Dulbecco's Modified Eagle Medium (DMEM) to which adequate antibiotics, 1% bovine serum albumin and 2 mg/ml collagenase was added. The cell suspension was incubated at 37°C under a constant gas phase of 95% 0_2 and 5% CO_2. Filtered cell suspension was allowed to stand at room temperature for 30-60 min. Floating brown adipocytes was aspirated and immediately used. Oxygen consumption was measured polorographically at 37°C using a 0_2 chamber attached to a 0_2 monitor. Basal 0_2 consumption was recorded for 5 min. Norepinephrine (NE), 1.0 μM and ethanol (0-4 mg/ml) were added alone or in combination to the cell suspension and the rate of 0_2 consumption was measured for 5 min thereafter. Apparent rate of 0_2 consumption in the absence of cells (i.e. blank) was subtracted from the rate obtained when the cells were present. Rate of 0_2 consumption calculated was expressed as nanomoles of 0_2/min/mg DNA of cell in cell suspension. Other details are given elsewhere (16). DNA estimations were done as described by Rafael and Vsiansky (17).

Statistical analysis of the data was carried out using one and two way analysis of variance and Duncan's range analysis for multiple comparisons between means. NCSS software was used for computation of the data. Results were considered statistically significant when $P<0.05$. All values are presented as means with their standard errors.

RESULTS

Ethanol given mice showed significantly reduced fluid intakes for the 10 day period ($P<0.006$). They also consumed less energy ($P<0.001$) as compared to their water drinking controls (Table I) for the same period of time. However, the body weight gains were comparable between the groups. BAT wet weight of the two groups were not significanly different from each other. These results are summarised in Table I.

Alcohol dehydrogenase (ADH) activity in the liver and BAT, expressed as nmol (NADH/min/organ is given Table II. BAT of mice showed traces of ADH activity (4.5 ± 1.5 nmol/min/organ) while in the liver it was quite high (879 ± 178 nmol/min/organ) in the water drinking mice. ADH activity in the ethanol given mice was further reduced in the BAT to a significant level. However, liver ADH activity in ethanol drinking mice was elevated but not significantly.

TABLE I : Body weight, energy and fluids intake and brown adipose tissue (BAT) wet weight of mice given only 10% ethanol or water for 10 days.

(Values are mean ± SE)

	Group	
	Water (n:6)	*10% ethanol (n:6)*
Body weight (g) :		
Initial	29.3 ± 0.4	30.0 ± 0.6
After 10 days	30.7 ± 0.6	31.4 ± 1.0
Weight gain	1.4 ± 0.7	1.4 ± 0.4
BAT wet weight (mg)	136.3 ± 9.2	114.3 ± 7.0
Energy intake (kJ) :		
Chow	924.3 ± 27.8	757.4 ± 15.8**
10% ethanol	–	7.3 ± 0.4
Total	924.3 ± 27.8	764.7 ± 16.1
Total fluid intake (ml)	83.5 ± 8.3	53.5 ± 2.7*

*P<0.006; **P<0.001; n : number of animals.

TABLE II : Alcohol dehydrogenase (ADH) activity expressed as nmol/min/organ in brown fat and liver tissues of mice drinking water or 10% ethanol for 10 days.

(Values are mean ± SE)

Tissue	*ADH activity (nmol/min/organ)* Group	
	Water (n:6)	*10% ethanol (n:6)*
Brown adipose tissue	4.5 ± 1.5	0.3 ± 0.1**
Liver	879.1 ± 178.1	1238.5 ± 213.7*

*Not significant, $F_{1,11}$ = 1.6, P < 0.2; **$F_{1,11}$ = 8, P < 0.02; n : number of animals.

Oxygen consumption of isolated brown adipocytes measured under several conditions are summarised in Table III. Basal 0_2 consumption of isolated BAT cells in the absence of ethanol was not significantly altered after adding ethanol upto a concentration of 4 mg/ml. Norepinephrine (NE) stimulated 0_2 consumption (index of BAT thermogenic capacity) was increased significantly (P<0.001) four times the basal value. Addition of ethanol did not further alter the NE stimulated 0_2 consumption of isolated cells (Table III).

TABLE III : Basal and Norepinephrine (NE) stimulated 0_2 consumption of isolated brown fat cells with or without ethanol.

(Values are mean ± SE)

0_2 consumption	*+Ethanol (mg/ml cell suspension)*			
(ml/min/mg DNA)-Ethanol	*0.5*	*1.0*	*2.0*	*4.0*
Basal	0.5 ± 0.1	1.3 ± 0.5	1.0 ± 0.3	1.0 ± 0.2	1.6 ± 0.5
NE stimulated	2.1 ± 0.6*	2.2 ± 0.5	2.0 ± 0.4	2.5 ± 0.6	2.4 ± 0.5

*F1,62 = 12.43, P < 0.001; n : six measurements from different samples for each concentration.

DISCUSSION

In the regulation of body weight and body temperature in both humans and animals, BAT has been shown to play a vital role (18, 19). Body weights are lowered as a part of adaptive response to decreased food intake (20, 21). Comparable body weight and wet weight of BAT of ethanol treated mice with that of water given controls after 10 days despite reduced energy intakes is contrary to the general response. This would probably indicate some other mechanism to maintain body weight in the growing young mice that is consuming less energy and fluids. It is likely that the ethanol drinking mice might retain more body water that would contribute for better body weights. It is also possible that the duration of treatment with 10% ethanol and the level of reduction of total energy intakes are not adequate enough to induce expected changes. It is equally possible that without apparent changes in wet weight of BAT pads functional or biochemical alterations may be occuring. Thus it is important to further evaluate body composition and BAT composition in detail in ethanol treated mice.

The occurence of high concentrations of ADH in the cytosol of liver parenchymal cells and its role in the conversion of ethanol to aldehyde and then to acetate and NADH and other aliphatic alcohols and certain steriods and fatty acids needs no emphasis (1, 4). However, the universal distribution of ADH in several other tissues raises question as to its physiological significance although in some tissues such as the retina and testis ADH has certain role to play (22, 23). The evidence available from this study for the existence of ADH in mice BAT and the direct measurement of basal and NE induced 0_2 consumption of isolated brown fat cells in the presence or absence of ethanol do not support the suggestion by Huttunen and Kortelainen

(6) in rat model that ADH may be important in the oxidation of ethanol in the brown fat of mice ingesting 10% ethanol. Our results are in accordance with very low levels of gastric ADH that do not contribute significantly to ethanol oxidation as demonstrated by Moreno as Pares (24).

It may thus be concluded that brown fat of mice like rat BAT contain traces of ADH activity. Ethanol consumption did not increase the enzyme contents in brown fat as it did in liver tissue. ADH in mice BAT is unlikely to play any significant role in the utilization and/or detoxification of ethanol.

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