Lack of adenosine deaminase deficiency in the mutant mouse *wasted*

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The possibility that the mutant mouse *wasted (wst/wst)* may serve as an animal model for studies of severe combined immunodeficiency disease (SCID) and the role of adenosine deaminase (ADA, EC 3.5.4.4) in adenosine metabolism were investigated. The specific activity of ADA in *wst/wst* compared with control mice was significantly lower by 26% in thymus, but significantly higher by 18% in spleen and 32% in cerebellum. V_{max} values of ADA in spleens were 43% higher in *wst/wst* mice and no changes were observed in K_m values. In contrast, the V_{max} of ADA was unchanged in erythrocytes from *wst/wst* mice, but the K_m for adenosine was significantly elevated. Thus, based on ADA measurements alone, it may be premature to consider *wst/wst* mice as a model for ADA deficiency and SCID in humans.

Adenosine deaminase *Adenosine* *Animal model* *Severe combined immunodeficiency disease*

1. INTRODUCTION

Adenosine deaminase (ADA, EC 3.5.4.4), a catabolic enzyme for adenosine and 2′-deoxyadenosine, plays a potentially multifaceted role in regulating cellular levels of purines. One approach to the study of ADA is through the use of animals deficient in this enzyme. Recently, a genetic strain of mice designated *wasted* was described carrying an autosomal recessive trait which in homozygous (*wst/wst*) animals results in neurological abnormalities, cerebellar degeneration [1], immunodeficiency, dramatically reduced tissue to body weight ratios for spleen, thymus and lymph nodes, and reduced numbers of lymphoid cells. While *wst/wst* mice were originally suggested as an animal model for ataxia telangiectasia [1,2], more recent work suggests closer similarities between the pathological changes in these mice and ADA-deficient humans suffering from severe combined immunodeficiency disease (SCID) [3]. On this basis and reported reductions of ADA activity in their erythrocytes [4], *wst/wst* mice were proposed as a possible animal model for SCID.

Our interest in ADA stems from the possibility that this enzyme may participate in regulating the neuromodulatory actions of adenosine in the CNS. In this regard, selective groups of parykarya and fiber systems immunoreactive for ADA have been identified in the rat and mouse brain [5–8]. Moreover, ADA activity in discrete CNS regions corresponds closely with the pattern of neural elements immunoreactive for ADA [9]. To evaluate the utility of *wasted* mice for the study of CNS systems containing ADA, we investigated whether the reported deficiency of ADA in these mice is exhibited by CNS tissue and re-examined ADA activity in some peripheral tissues.

2. MATERIALS AND METHODS

2.1. *Animals*

Heterozygote breeding pairs of C57 BL/6 × C3 HeB/FeJ/F1 hybrid mice carrying the autosomal

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recessive gene *wasted* (*wst/wst*) were obtained from the Jackson Laboratory (Bar Harbor, ME). Typically, the homozygous *wst/wst* animals exhibit progressive deterioration in health after weaning and die at approx. 30 days of age [2]. When *wst/wst* animals showed clear signs of wasting (23–27 days of age), the entire litter was killed by cervical fracture and whole brain, hypothalamus, cerebellum, thymus and spleens were dissected, frozen on dry ice and stored at −80°C. Blood obtained by cardiac puncture using heparinized syringes was centrifuged at 3000 × *g* for 10 min and erythrocytes were stored at −80°C.

2.2. *ADA assay*

As described in [9], tissue samples were homogenized with a Polytron in ice-cold 50 mM Tris buffer, pH 7.0, containing 10 mM $MgCl_2$. 50 μl aliquots of homogenate were added to reaction vessels containing concentrations of adenosine ranging from 7.8 to 500 μM. Following incubation at 37°C for 5–30 min, the reaction was stopped by adding 10 μl of 20% trichloroacetic acid. The samples were neutralized and 25 μl aliquots of the aqueous phase were chromatographed using reverse-phase HPLC [9]. ADA activity was expressed as nmol product formed (inosine plus hypoxanthine)/mg protein per mg wet wt tissue or total activity per tissue. The K_m and V_{max} values were determined using computerized, weighted least-squares regression analysis.

3. RESULTS

The body weights of *wst/wst* mice, 7.7 ± 0.2 g, were significantly less than those of their littermate controls, 14.3 ± 0.4 g ($P < 0.001$). In *wst/wst* mice, individual tissue weights, expressed as percent of control values, were 92% for whole brain, 29% for thymus, 27% for spleen and 91% for cerebellum. The weights of hypothalamus were unchanged. As shown in table 1, ADA activity (per mg tissue) in whole brain and cerebellum from *wst/wst* mice was 22 and 32% higher, respectively. These increases were also evident when the data were expressed as total tissue activity; higher by 17% in brain and 26% in cerebellum. A non-significant increase of 11% was found in hypothalamus. In thymus and spleen of *wst/wst*

Table 1

Adenosine deaminase activity in tissues from control (C) and *wasted* (W) mice

Tissue		ADA activity (nmol/30 min)			Tissue wt (g)	
		Per mg protein	Per mg tissue	Total in tissue		
Whole brain	(C)	86 ± 5	8 ± 0.2	3265 ± 129	415 ± 10	(9)
	(W)	108 ± 15	10 ± 1.1	3916 ± 440[a]	383 ± 2[a]	(4)
Hypothalamus	(C)	136 ± 6	10 ± 0.6	109 ± 6	10 ± 0.5	(15)
	(W)	139 ± 19	11 ± 1.0	122 ± 12	11 ± 0.8	(7)
Cerebellum	(C)	81 ± 4	7 ± 0.4	405 ± 28	58 ± 1	(10)
	(W)	117 ± 4[c]	10 ± 0.4[c]	548 ± 34[b]	53 ± 1[a]	(7)
Thymus	(C)	3103 ± 99	475 ± 18	37467 ± 2303	76 ± 4	(15)
	(W)	2891 ± 187	377 ± 28[b]	6016 ± 824[c]	22 ± 6[c]	(6)
Spleen	(C)	1221 ± 56	170 ± 10	9183 ± 672	60 ± 6	(11)
	(W)	1699 ± 64[c]	207 ± 12[a]	3226 ± 400[c]	16 ± 2[c]	(6)

[a] $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.001$

Values represent means ± SE of the number of determinations indicated in parentheses. Statistical analyses were conducted using a 2-tailed Student's *t*-test

mice, the ADA activity (per mg tissue) was reduced by 26% in thymus, but increased by 18% in spleen. Similar patterns were observed when ADA activity was expressed as product formed/mg protein. A slightly different picture emerged when the data were calculated as total activity per organ since spleen and thymus weights were severely reduced and this was reflected by the 84 and 77% reduction, respectively in the total ADA activity of these organs. V_{max} values of ADA activity in spleens from *wst/wst* mice (fig.1) were significantly increased by 43% ($P < 0.01$), and no significant differences were found in the K_m values. For blood (fig.2), no differences in V_{max} values were found, however the K_m values from *wst/wst* mice were significantly ($P < 0.01$) higher by 78% (lower affinity).

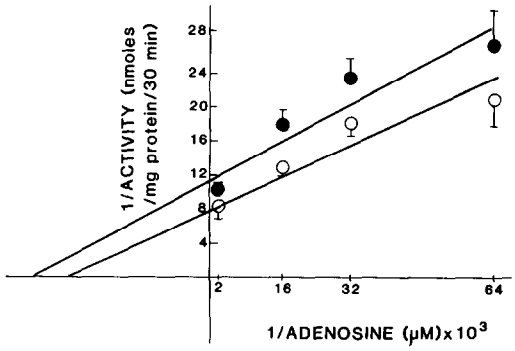

Fig.1. Double-reciprocal plot of ADA activity in whole spleen homogenates vs adenosine concentration from control (○) and *wst/wst* (●) mice. Symbols represent means ± SE of three separate experiments. Regression lines were drawn according to weighted least-squares analyses. V_{max} (nmol/mg protein per 30 min) and K_m (μM) values for controls were 1329 ± 51 and 23.7 ± 2.8, and for *wst/wst* mice, 1900 ± 23 and 29.4 ± 3.2, respectively.

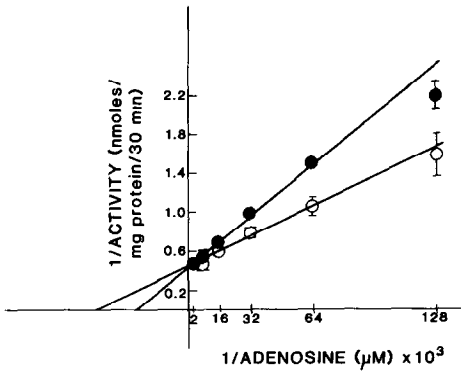

Fig.2. Double-reciprocal plot of ADA activity in erythrocytes vs adenosine concentration from control (○) and *wst/wst* (●) mice. Symbols represent means ± SE of four separate experiments for control and three experiments for *wst/wst* mice. Points lacking error bars indicate that the SE values did not exceed the size of the symbols. Regression lines were drawn according to weighted least-squares analyses. V_{max} (nmol/mg protein per 30 min) and K_m (μM) values were 64.3 ± 1.4 and 20.8 ± 1.6 for controls, and 61.1 ± 3.0 and 37.1 ± 3.0 for *wst/wst* mice, respectively.

4. DISCUSSION

The reduced tissue weights in *wst/wst* mice necessitated the calculation of ADA activity both in terms of specific activity (per mg tissue and protein), and total activity in the tissues. Based on specific activity measurements, significant increases in ADA activity were observed in whole brain, cerebellum, and spleen, and a significant decrease was found in thymus of *wst/wst* mice. However, based on total ADA activity, significant increases were noted in whole brain and cerebellum, whereas thymus and spleen activity were both dramatically reduced. The reduced weight and greater total ADA activity in brain tissues indicate an elevated absolute amount of enzyme activity in these tissues. The functional significance of these changes in brain and peripheral tissues may become evident once the cell types within which they occur are determined.

In agreement with the preliminary finding of Abbott et al. [4], the total ADA activity in thymus from *wst/wst* mice was 17% of control levels. However, this reflects the large tissue deterioration which occurs in *wst/wst* mice since much smaller reductions were observed in the specific activity of ADA. Moreover, the specific activity and V_{max} of ADA in spleen of *wst/wst* mice were significantly higher than controls despite a large loss of total activity. Previously, K_m and V_{max} values for ADA activity in erythrocytes from *wst/wst* mice were found to be 51 and 50% of control values, respectively [4]. In the present study, however, the K_m value for erythrocytes from *wst/wst* mice, unlike the unchanged values in spleen, was almost twice that of controls and no changes in V_{max} were found. The differences in the results of the present

study and that of Abbott et al. [4] may have been due to different procedures used in measuring ADA activity, or more likely the cross-breeding of their *wasted* animals with ones carrying the visible-marker gene *ragged*. This intercrossing led to *ragged-wst/ragged-wst* mice which were almost completely naked and which, unlike the original *wasted* breeding mice supplied by Jackson Laboratories used here and by others [1–3], died before weaning. Thus, a genetic variance may have been introduced which accounts for the disparate results.

Since *wasted* mice were proposed as an animal model for SCID, it is important to note that immunological deficiency, which leads to death in children suffering from SCID, appears only in those individuals completely lacking ADA activity in erythrocytes and lymphoid cells and only an estimated 50–60% of patients with autosomal recessive inherited SCID present with ADA deficiency [11]. Moreover, children completely lacking in erythrocyte ADA activity yet retaining sufficient activity in other tissues (e.g. lymphocytes) appear to be immunologically normal [11–13]. Since ADA levels in blood and lymphoid tissues of *wasted* mice are not completely absent, and in many tissues are actually increased, these mice are unlikely to serve as a useful model for SCID or for the study of ADA function in the CNS.

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