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Developmental expression and corticosterone inhibition of adenosine deaminase activity in different tissues of mice

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Abstract

The activity expression and corticosterone inhibition of adenosine deaminase (ADA) were studied in the spleen, stomach, and liver of mice at various postnatal ages. The specific activity of ADA is very low in the spleen and stomach of 5- and 10-day-old mice, and increases significantly (2.5- to 3.0-fold) in 20- and 30-day-old animals. Its level shows a further increase in the spleen of 60-day-old mice while stomach increase of ADA is not significant. In contrast, the activity of ADA is significantly higher in the liver of 5- and 10-day-old mice, decreases markedly (2.5-fold) in 20- and 30-day-old animals and shows a sharp increase in the liver of 60-day-old mice. Corticosterone administration brings a marked inhibition in the activity of ADA at all ages studied in the spleen and stomach whereas it inhibits the liver ADA only at 30 and 60 days postnatal age. These findings suggest an age- and tissue-specific expression of ADA activity and also indicate corticosterone as an inhibitory regulator of this enzyme.

Keywords: Adenosine deaminase; Mice; Development; Corticosterone

1. Introduction

Adenosine deaminase (ADA; EC 3.5.4.4) catalyzes the irreversible hydrolytic deamination of adenosine and 2′-deoxyadenosine to inosine and 2′-deoxyinosine,

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respectively [1,2]. Its function is critical to control the concentration of adenosine and 2′-deoxyadenosine in a variety of systems including immunological [3], neurological [4], and vascular systems [5]. ADA is phylogenetically ubiquitous and widely distributed throughout mammalian tissues and the level of ADA varies markedly among different tissues and within the same tissues from different species [1]. The mouse ADA gene has been cloned and its promoter has GC rich sequences with multiple transcription CAAT boxes, indicative of its similarity with constitutively expressed housekeeping enzyme [6,7]. However, it is difficult to accept an ubiquitous housekeeping role for ADA in view of its many fold varied activities in different peripheral tissues and its increase in activity by 10- to 30-fold during cellular differentiation [8]. The SP1, a GC box binding protein, is essential for both enhancer-mediated and basal activation of the TATA-less human ADA promoter [9]. The levels of ADA mRNA are also posttranscriptionally regulated with parallel levels of ADA activity [10].

Patients with ADA deficiency lack both T and B lymphocyte mediated functions and exhibit a severe combined immunodeficiency (SCID) disorder [11]. Plasticity in ADA expression is further suggested by its increased activity in patients with congenital hypoplastic anemia [12] and hereditary hemolytic anemia [13]. During development, physiological adjustments take place in different tissues as an adaptation to the changing demands made upon them and these adjustments are accomplished by sequential activation and repression of genes causing alterations in the levels of the various enzymes involved [13,14]. Development of the lymphatic system and immunity is one of the most important physiological adjustments which an animal has to undergo in order to develop a remarkable immunological competence in growing animals. In this paper, prompted by the role of ADA in lymphatic and immune systems and also by the role of corticosterone as an immunosuppressor, we studied the activity expression of ADA and also the effect of corticosterone on this enzyme in the spleen (lymphoid), stomach, and liver (gastrointestinal) of female mice at various postnatal ages.

2. Materials and methods

2.1. Materials

Female Swiss albino mice (Balb/c strain) of five different age groups (5-, 10-, 20-, 30-, and 60-day-old) were used. They were maintained under normal laboratory conditions at 24 ± 2°C and fed with standard pellet diet (Amrut Laboratory, Pune) and water ad libitum. All the chemicals used were of analytical grade and biochemicals were obtained from Sigma Chemical Co., St. Louis, MO.

2.2. Preparation and assay of ADA

Animals were killed by cervical dislocation at a fixed time of the day (14:00 h), their spleen, stomach, and liver were taken out, washed in chilled normal saline (0.9% NaCl), blotted dry, and stored at −70°C until use. A 20% (w/v) homogenate of spleen and liver and 10% homogenate of the stomach were prepared in ice-cold 100 mM sodium citrate buffer, pH 6.0 having 0.25 M sucrose. Each homogenate

was centrifuged at 27 500 × *g* for 60 min at 0°C in a Hitachi Model CR20B2 high speed refrigerated centrifuge. The supernatant thus obtained was used for the assay of ADA.

ADA activity was measured spectrophotometrically in a Hitachi Model U-2000 spectrophotometer by the method of Kalchar [15] and Yoshida and Aikawa [16] with certain modifications. Initial reaction rates were determined from the decrease in the absorbance at 265 nm. The standard assay was carried out at 25°C in 3.0 ml of 100 mM sodium citrate buffer, pH 6.0 with 100 μM adenosine and a suitable amount of enzyme preparation (50 μl) which gave a linear decrease at A_{265}. The protein concentration of the enzyme preparation was determined by the method of Bradford [17]. The activity of ADA was expressed as units (μmol adenosine deaminated per min) per mg protein. The data were statistically analyzed [18]. The level of significance (*P*-value) between two sets of data was calculated according to student's *t*-test.

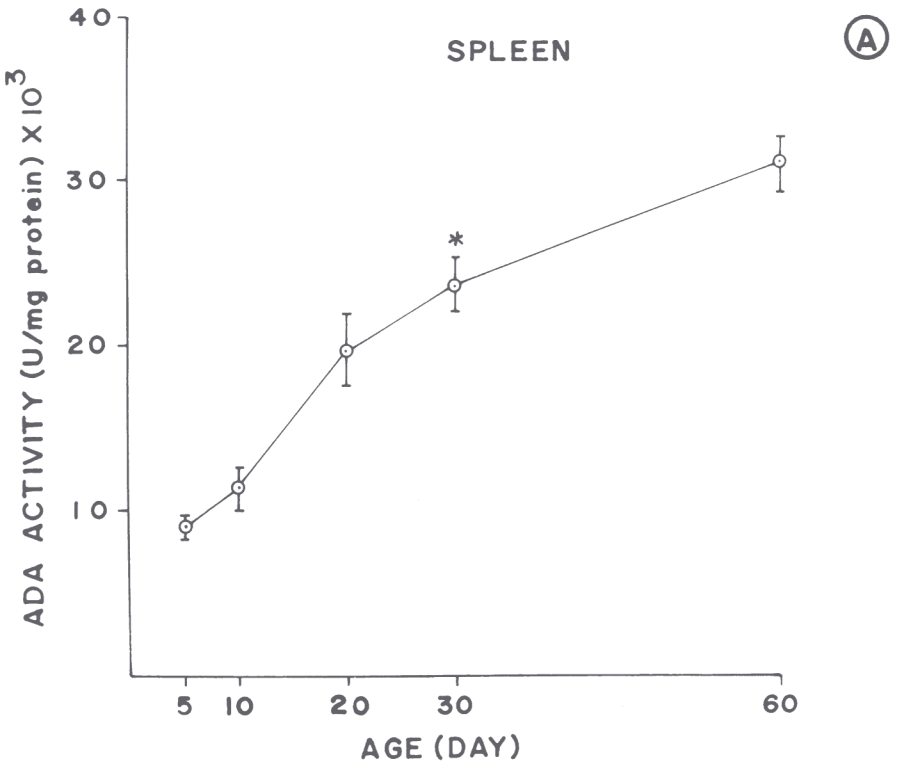

Fig. 1. Activity of adenosine deaminase (ADA) in the spleen (A), stomach (B), and liver (C) of normal mice of different postnatal ages. Fractionation and assay conditions are described in section 2.1. and 2.2. Values are means for 4–5 mice in each age group. Bars, S.D. All the observed differences are statistically significant by student's *t*-test except the asterisk.

2.3. Corticosterone treatment

Several experiments were done to find out the time- and dose-response of ADA towards corticosterone in mice of various ages. Maximum response of the enzyme was obtained 6 h after corticosterone administration at a dose of 1.0 mg/100 g body weight. Corticosterone was administered (09:00 h) in 300 μl normal saline having 6% ethanol intraperitoneally (i.p.) and control animals received equal amount of saline and ethanol solution. All the animals were killed after 6 h of hormone administration and tissues were taken out, washed in normal saline, blotted dry and used for further analysis.

3. Results and discussion

Both T and B lymphocytes appear to be extremely sensitive to adenosine. In normal individuals, relatively high ADA activity in lymphoid tissues appears to maintain low extracellular levels of adenosine, permitting lymphocyte survival [19]. Lack of ADA activity leads to accumulation of *S*-adenosylhomocysteine and dATP and arrest of lymphocytes blastogenesis. Many-fold action of adenosine appears to be mediated via specific cell-surface receptors linked to adenylate cyclase. These

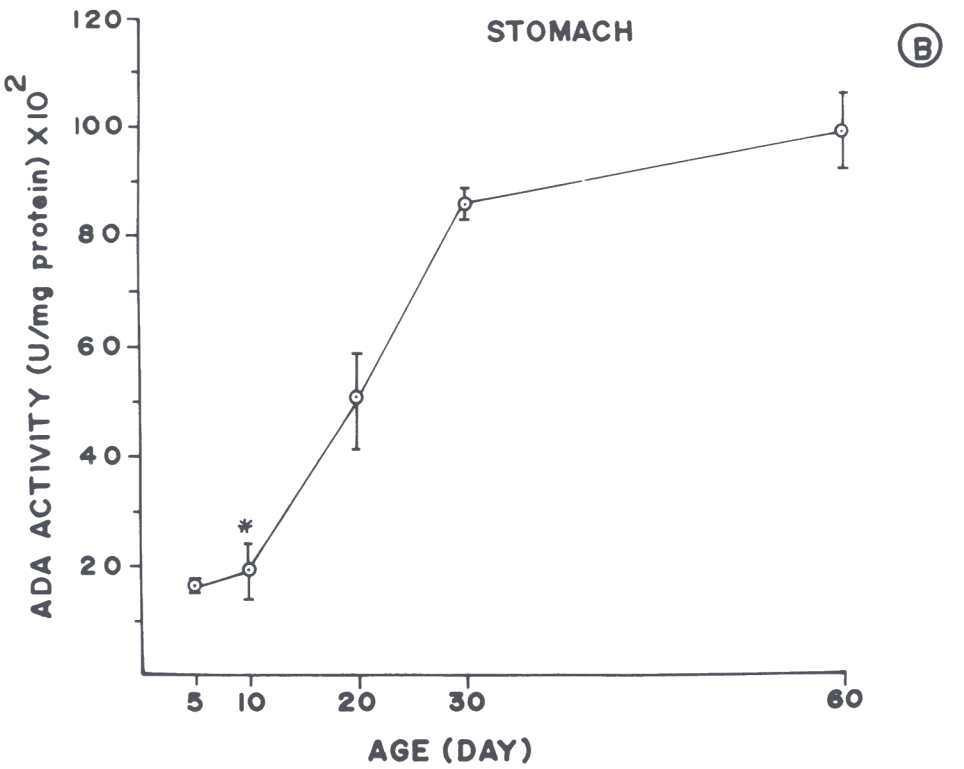

Fig. 1. (B).

receptors are of two types: A1, inhibitory and A2, stimulatory to adenylate cyclase [20,21]. Thereby, they influence the intracellular synthesis of cAMP which modulates the cellular response. Keeping in mind the role of adenosine and its deaminating enzyme in the immune (spleen) system and gastrointestinal tract (stomach, liver), we chose to study the normal endogenous level of ADA in these tissues at various postnatal ages. We also report here the effect of corticosterone, an immunosuppressive agent, on the activity of this enzyme.

The normal endogenous level of ADA activity (Unit/mg protein) is shown in Fig. 1A–C for spleen, stomach, and liver of female mice at various postnatal ages. Our data indicate a high level of ADA in stomach as compared to spleen and liver. The lowest activity of ADA in the liver indicates its greater physiological role in lymphoid organs such as spleen and stomach [22]. A higher level of ADA in stomach may also function to ensure that dietary sources of adenosine do not exert unwanted physiological effects [22]. The activity of ADA is very low in the spleen and stomach of 5- and 10-day-old mice, increases significantly (2.5- to 3-fold) in 20- and 30-day-old mice and shows a further slight increase in 60-day-old mice. The increase in the activity of this enzyme may help these animals to cope with an increasing need for immunological competence during the post-weaning period. Higher levels of ADA may maintain lower adenosine levels ensuring better

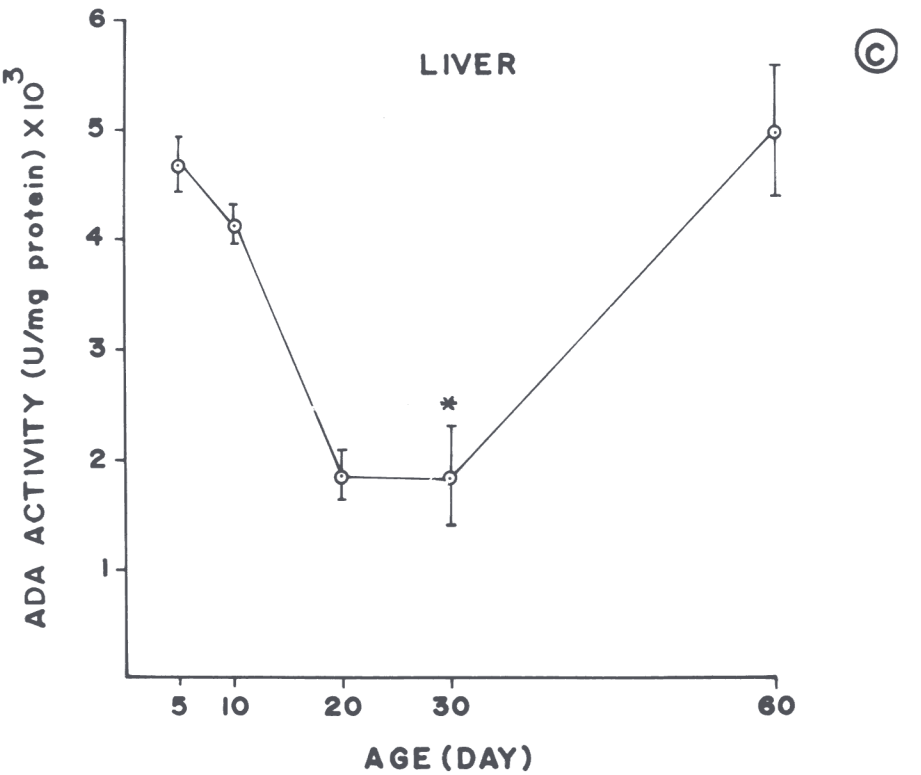

Fig. 1. (C).

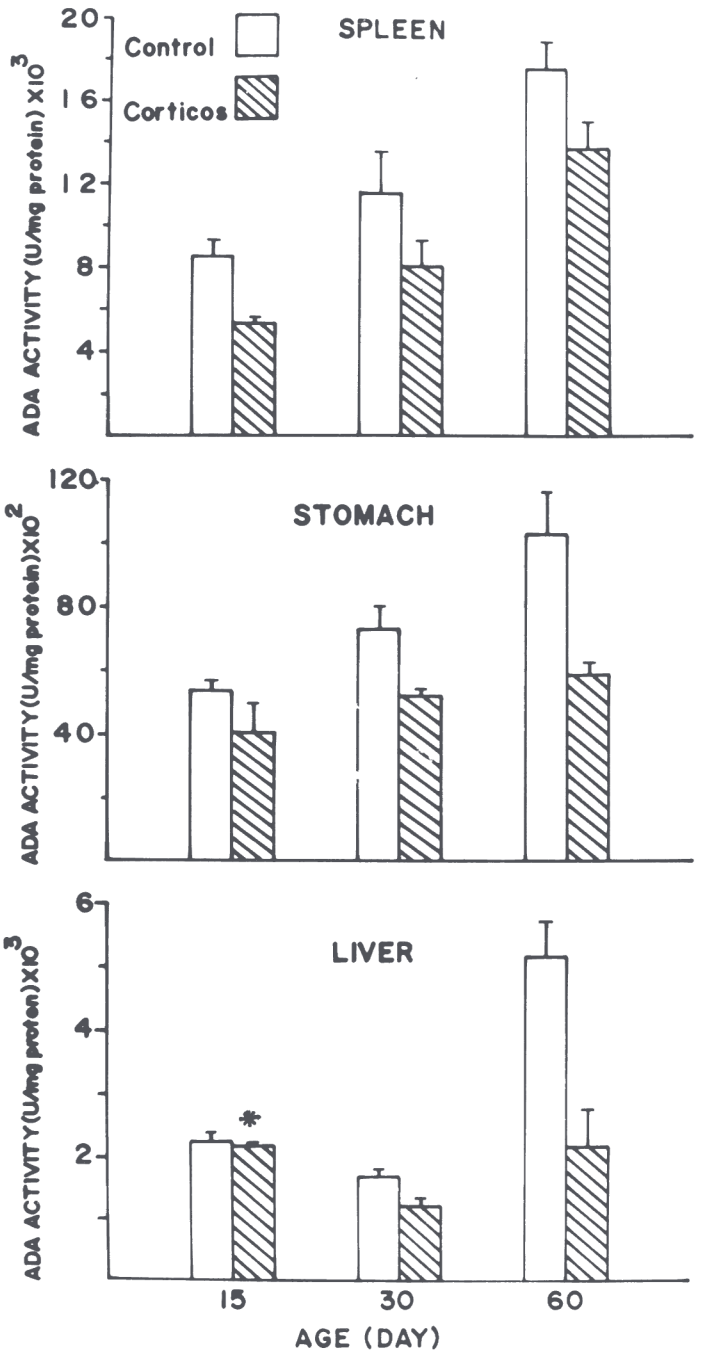

Fig. 2. Effect of corticosterone (corticos) on the activity of adenosine deaminase (ADA) in the spleen, stomach, and liver of mice at various postnatal ages. Experimental conditions are described in section 2.3. Values are means for 4–5 mice in each age group. Bars, S.D. Statistical analysis is same as Fig. 1.

lymphocytes survival during postnatal development of mice. In contrast, liver ADA shows a different pattern of expression (Fig. 1C). Its activity is very high at 5 and 10 days postnatal age, decreases significantly (2- to 3.5-fold) in 20- and 30-day-old animals and shows a sharp increase in the liver of 60-day-old mice. The different pattern of hepatic ADA expression may indicate a different role for ADA in the liver during postnatal development of mice.

Our results also indicate that corticosterone inhibits the activity of ADA in all the three tissues studied (Fig. 2). The magnitude of inhibition shows tissue- and age-specificity. In general, the magnitude of inhibition is more pronounced in the stomach and liver of mice at 60 days postnatal age whereas the spleen shows more inhibition of ADA at 15 days postnatal age. Corticosteroids are known to exert a multitude of effects on a variety of systems [23]. They influence the cellular responses by interacting with cognate intracellular receptors and by the binding of these hormone-receptor complexes to specific DNA sequences termed hormone responsive elements (HREs), usually located 100–300 bp upstream from the RNA polymerase start site, and ultimately causing a change in transcription of specific genes [24,25]. The tissue- and age-specific changes in the corticosterone inhibition of ADA activity may be due to changes in hormone receptor and/or in the post-receptor events specific to different tissues studied at various postnatal ages in mice [26,27]. Inhibitory action of corticosterone on ADA activity may be correlated with the greater accumulation of its substrates adenosine and deoxyadenosine which produce lymphoid toxicity and suppress immune responses [3,28]. More investigation is needed on corticosterone inhibition of ADA activity to understand better the mechanism of inhibition and also to suggest a use for this hormone in the management of ADA related disorders.

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