J. Biochem. Mol. Biol. & Biophys., Vol. 1, pp. 125–132
Reprints available directly from the publisher
Photocopying permitted by license only

Developmental and Hormonal Regulation of Intestinal Adenosine Deaminase in Mice*

LISAM SHANJUKUMAR SINGH and RAMESH SHARMA†

Department of Biochemistry, North Eastern Hill University, Shillong 793 022, India

(Received 1 October 1996; Revised 3 April 1997; Accepted 10 April 1997)

The normal endogenous level of adenosine deaminase (ADA) and its regulation by corticosterone and dibutyryl-cAMP were studied in the small intestine of mice at various postnatal ages. The activity (U/mg protein) of ADA is very low at day 5, increases slightly at day 10 and reaches a peak value at day 20 in the small intestine of mice. The level of ADA decreases significantly thereafter in 30- and 60-day old mice similar to a level of 10-day old animal. Administration of corticosterone inhibits the activity of ADA in all the postnatal ages studied with a maximum inhibition in the intestine of 60-day old mice. Adrenalectomy, on the other hand, increases the activity of ADA in this tissue of mice. In contrast, dibutyryl-cAMP increases intestinal ADA activity in all the ages studied. Prior administration of actinomycin D has no effect in blocking the dibutyryl-cAMP-dependent increase in ADA activity. These findings indicate an age-specific expression of intestinal ADA activity during postnatal development of mice. Our findings also entail corticosterone as an inhibitory and dibutyryl-cAMP as a stimulatory signal for ADA activity.

Keywords: Adenosine deaminase, Small intestine, Development, Corticosterone, Bt_2-cAMP

INTRODUCTION

Adenosine deaminase (ADA; EC 3.5.4.4) catalyzes the irreversible hydrolytic deamination of adenosine and 2′-deoxyadenosine to inosine and 2′-deoxyinosine, respectively [1]. The function of ADA is critical in controlling the level of adenosine and 2′-deoxyadenosine in a variety of organ systems [2]. ADA is phylogenetically ubiquitous and widely distributed throughout mammalian tissues. The mouse ADA gene promoter is highly GC-rich, devoid of consensus TATA and CAAT boxes, indicating its similarity with constitutively expressed housekeeping enzymes [3]. However, ADA level varies markedly in different peripheral tissues and shows elevation of upto 10- to 30-fold during cellular differentiation [2,4]. A GC-box binding protein (SP1) is essential for both enhancer-mediated and basal activation of human ADA promoter [5]. The levels of ADA mRNA are also regulated post-transcriptionally with parallel levels of ADA activity [6].

*This work was presented at the 12th International symposium of FAOBMB held at Tokushima, Japan.

†Corresponding author. Tel.: 91-0364-250107. Fax: 91-0364-250076.

Lack of ADA has been correlated with the failure of both T and B lymphocyte mediated functions and associated with severe combined immunodeficiency (SCID) disorder [7]. Increased level of ADA activity in patients suffering from congenital hypoplastic anemia, hereditary hemolytic anemia, leukemia and hepatitis further suggests the plasticity in ADA expression [8]. In adult mice, the highest level of ADA is present in the keratinized squamous epithelium that lines the alimentary canal, where the enzyme accounts for as much as 20% of the total soluble protein [9,10]. Adenosine, a natural substrate of ADA, is believed to influence immunological stimulation of Cl^- secretion in intestinal epithelium which results in movement of isotonic fluid into the lumen, and help to hydrate the mucosal surface [11]. Keeping in view the role of ADA and its natural substrates in the physiology of the gastrointestinal tract, we herein studied the developmental expression of ADA and the effects of corticosterone, an immunosuppressor and dibutyryl-cAMP, an immunoinducer [12] on ADA in the small intestine of female mice at various postnatal ages.

MATERIALS AND METHODS

Materials

Female Swiss albino mice (Balb/c strain) of five different age groups (5-, 10-, 20-, 30- and 60-day old) were used. The animals were maintained under normal laboratory conditions at $25 \pm 1°C$ on a 12/12 h light/dark period and fed with a 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., USA.

Preparation and Assay of ADA

Animals were killed by cervical dislocation at a fixed time of the day (14:00 h), their small intestinal tubes were taken out, washed in chilled normal saline (0.9% NaCl) and blotted dry. A 20% (w/v) homogenate of the intestine was prepared in ice-cold 100 mM sodium citrate buffer, pH 6.0 containing 0.25 M sucrose. Each homogenate was centrifuged at $27,500 \times g$ for 60 min at 2°C. The supernatant thus obtained was used for the assay of ADA. The activity of ADA was measured spectrophotometrically in a Hitachi Model U-2000 spectrophotometer by the method of Kalchar [13] and Yoshida and Aikawa [14] with certain modifications. Initial reaction rates were determined from the decrease in absorbance at 265 nm. The standard assay was carried out at 25°C in 3 ml of 100 mM sodium citrate buffer, pH 6.0 with 100 μM adenosine and 50 μl of suitably diluted enzyme preparation that gave a linear decrease in absorbance at 265 nm.

Protein concentration of enzyme preparation was determined by the method of Bradford [15] using BSA as standard. The activity of ADA was expressed as units (μmol adenosine deaminated per min) per mg protein. The data were statistically analyzed and the level of significance (p-value) between two sets of data was calculated according to Student's t-test.

Hormonal Treatment

A dose and time response of ADA activity towards corticosterone were standardized as described earlier [4]. It was administered intraperitoneally at a dose of 1.0 mg/100 g body weight in 0.3 ml normal saline having 6% ethanol. Control animals received equal amounts of saline and ethanol solution. All the animals were killed after 6 h of treatment. A group of female mice of 30-day was bilaterally adrenalectomized and kept for 7 days on normal saline instead of water together with normal pellet diet. Thereafter, animals were killed and small intestine was used for the assay of ADA. Dibutyryl-cAMP (Bt_2-cAMP), a membrane permeable analog of cAMP, was administered intraperitoneally (09:00 h) at different doses (0.1, 0.3, 0.6 and 1.5 mg/100 g body weight) in 0.3 ml of normal saline. Control

animals received equal amounts of saline only. The animals were killed after 3/6 h of Bt_2-cAMP administration. Actinomycin D, a transcriptional inhibitor, was administered at a dose of 10 μg/100 g body weight in 0.3 ml of normal saline intraperitoneally, 1 h prior to Bt_2-cAMP administration.

RESULTS

Normal Endogenous Level of ADA Activity

The normal endogenous level of ADA activity (U/mg protein) is lowest in the small intestine of 5-day old mice (Fig. 1). It increases (3-fold) at day 10 and peaks (7–8 fold) at day 20 of postnatal development. Thereafter, the activity of ADA decreases markedly at day 30 (1.2-fold) and 60 (5-fold). The level of ADA activity at day 60 remains almost similar to that of day 10. The activity expression of ADA shows a bell shape pattern in the small intestine during postnatal development, exhibiting a peak value in 20-day old mice.

Effect of Corticosterone on ADA Activity

Effect of corticosterone on ADA was studied in the small intestine of mice at three postnatal ages (15-, 30-, and 60-day old). Our results indicate that corticosterone inhibits significantly the activity of intestinal ADA in all three postnatal ages (Fig. 2). The magnitude of inhibition is more pronounced at day 60 (60%), whereas, at day 15 and 30 inhibition of ADA activity is 25 and

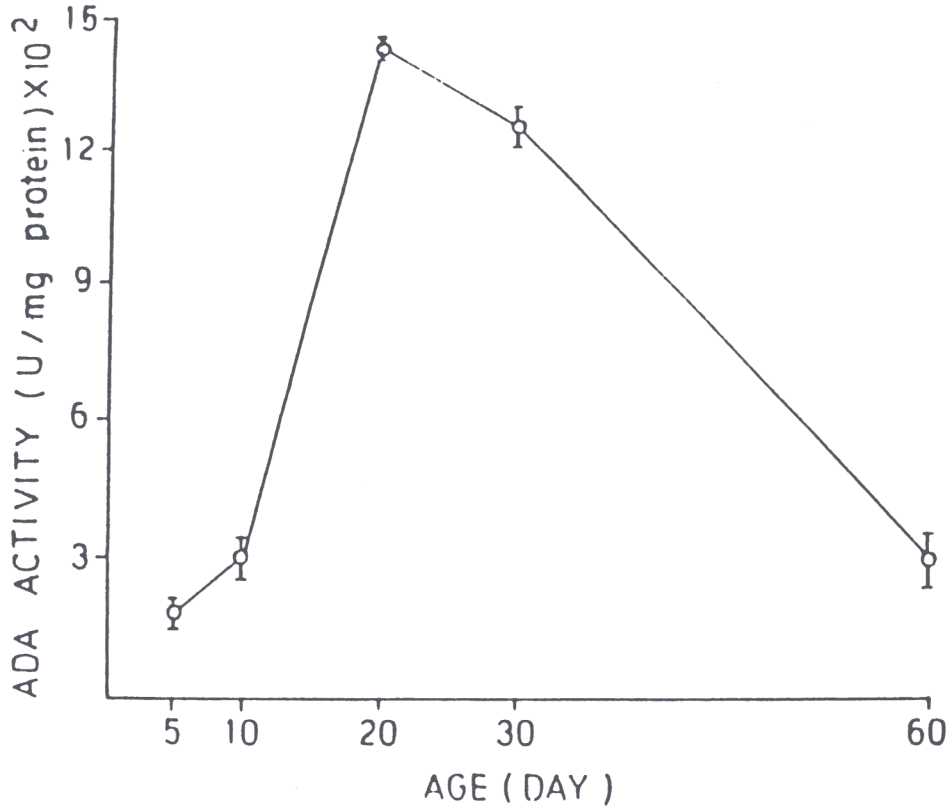

FIGURE 1 Activity of adenosine deaminase (ADA) in the small intestine of normal female mice of different postnatal ages. Fractionation and assay conditions are described in Materials and Methods section. Values are means for 4–5 mice in each age group. Bars represent standard deviation. All the points are statistically significant ($P < 0.01$) as compared to day 5 value.

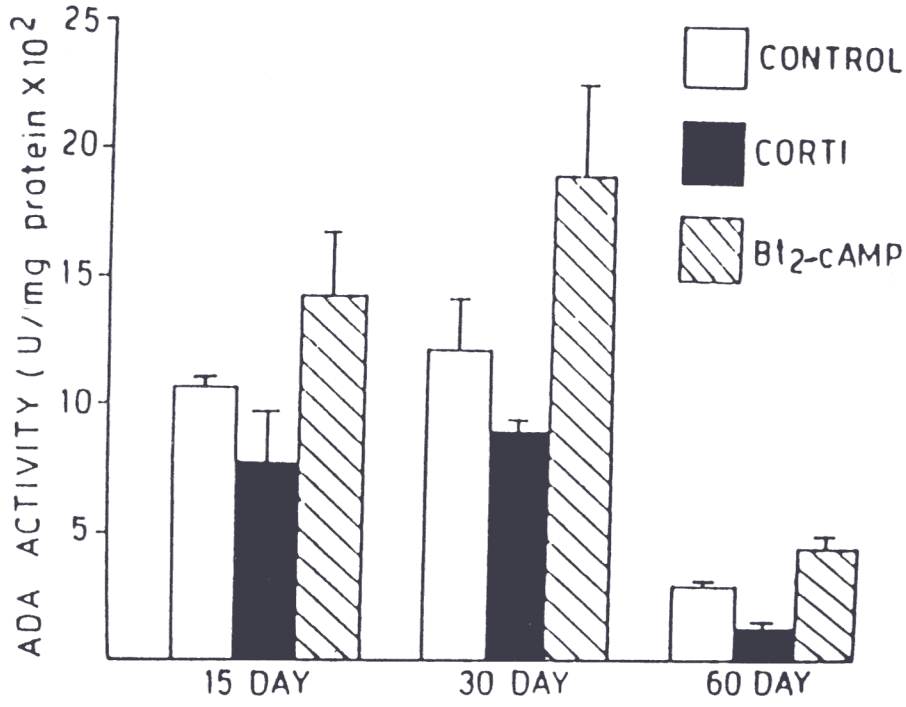

FIGURE 2 Effects of corticosterone (CORTI) and dibutyryl-cAMP (Bt_2-cAMP) on the activity of adenosine deaminase (ADA) in the small intestine of mice at various postnatal ages. Hormonal treatments and the other experimental conditions are described in Materials and Methods section. Values are means for 4–5 mice in each age group. Bars represent standard deviation. All the observed differences are statistically significant ($P < 0.01$) as compared to control.

30%, respectively. To ascertain the endogenous role of corticosterone inhibition of intestinal ADA activity, we studied the effect of adrenalectomy on the activity of ADA in the small intestine of 30-day old mice. Adrenalectomy, significantly increased (60%), the level of ADA as compared to control corroborating the finding of corticosterone inhibition of enzyme activity (Fig. 4).

Effect of Dibutyryl-cAMP on ADA Activity

In order to find out a stimulatory regulator of ADA activity, we studied the effect of dibutyryl-cAMP on the activity of intestinal ADA. Results indicate that Bt_2-cAMP enhances the activity of intestinal ADA in all the age groups studied (Fig. 2). It also exhibited a dose- and time-dependent enhancement in ADA activity (Figs. 3A and B). Maximum response of Bt_2-cAMP was obtained at a dose of 0.6 mg/100 g body weight after 6 h of treatment. To assess the mode of ADA activity enhancement, actinomycin D, a transcriptional inhibitor, was used with Bt_2-cAMP. Administration of actinomycin D prior to Bt_2-cAMP injection does not inhibit the increase in level of ADA activity by Bt_2-cAMP, negating its role at transcriptional level (Fig. 4).

DISCUSSION

Adenosine deaminase has gained considerable importance as it controls the level of adenosine and deoxyadenosine. The physiological consequences of these metabolites are well documented in individuals with a genetic defect in this enzyme [7]. ADA happens to be present in high amounts in lymphoid tissues of normal individuals so as to maintain low concentration of adenosine and deoxyadenosine, permitting better lymphocyte

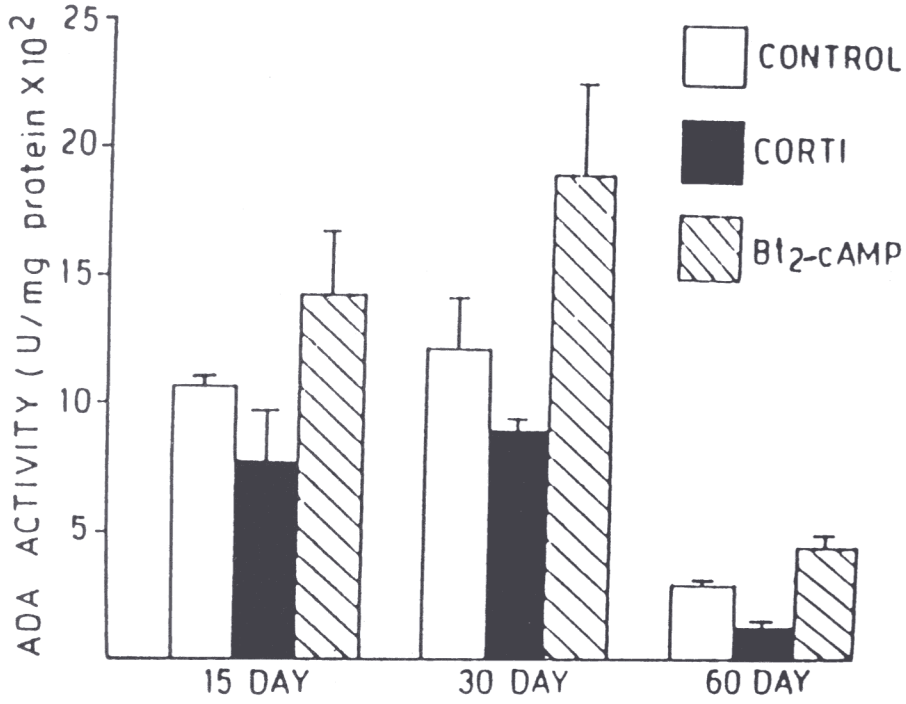

FIGURE 2 Effects of corticosterone (CORTI) and dibutyryl-cAMP (Bt_2-cAMP) on the activity of adenosine deaminase (ADA) in the small intestine of mice at various postnatal ages. Hormonal treatments and the other experimental conditions are described in Materials and Methods section. Values are means for 4–5 mice in each age group. Bars represent standard deviation. All the observed differences are statistically significant ($P < 0.01$) as compared to control.

30%, respectively. To ascertain the endogenous role of corticosterone inhibition of intestinal ADA activity, we studied the effect of adrenalectomy on the activity of ADA in the small intestine of 30-day old mice. Adrenalectomy, significantly increased (60%), the level of ADA as compared to control corroborating the finding of corticosterone inhibition of enzyme activity (Fig. 4).

Effect of Dibutyryl-cAMP on ADA Activity

In order to find out a stimulatory regulator of ADA activity, we studied the effect of dibutyryl-cAMP on the activity of intestinal ADA. Results indicate that Bt_2-cAMP enhances the activity of intestinal ADA in all the age groups studied (Fig. 2). It also exhibited a dose- and time-dependent enhancement in ADA activity (Figs. 3A and B). Maximum response of Bt_2-cAMP was obtained at a dose of 0.6 mg/100 g body weight after 6 h of treatment. To assess the mode of ADA activity enhancement, actinomycin D, a transcriptional inhibitor, was used with Bt_2-cAMP. Administration of actinomycin D prior to Bt_2-cAMP injection does not inhibit the increase in level of ADA activity by Bt_2-cAMP, negating its role at transcriptional level (Fig. 4).

DISCUSSION

Adenosine deaminase has gained considerable importance as it controls the level of adenosine and deoxyadenosine. The physiological consequences of these metabolites are well documented in individuals with a genetic defect in this enzyme [7]. ADA happens to be present in high amounts in lymphoid tissues of normal individuals so as to maintain low concentration of adenosine and deoxyadenosine, permitting better lymphocyte

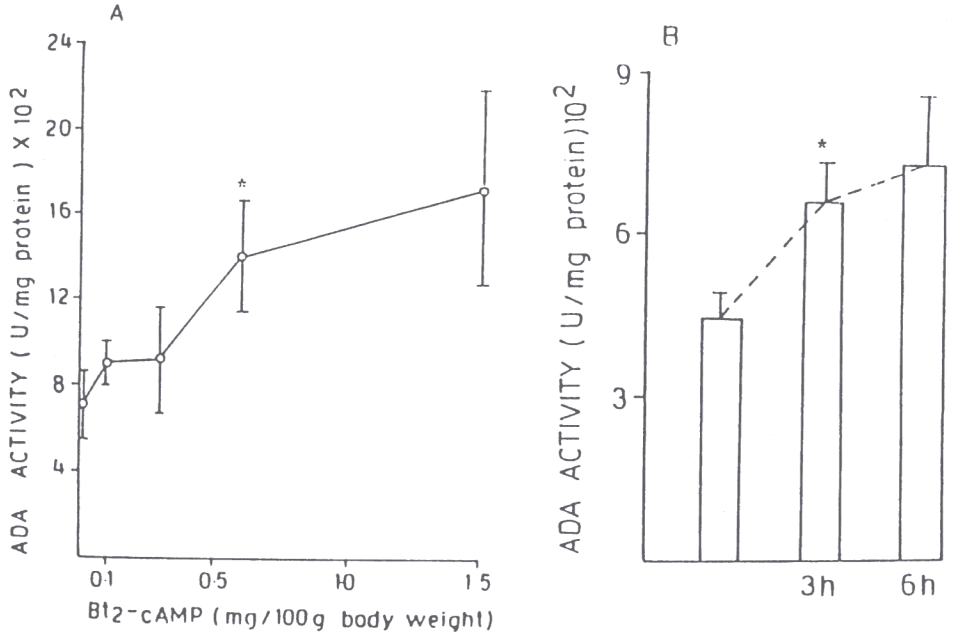

FIGURE 3 Dose-response (A) and time course (B) of Bt_2-cAMP induction of adenosine deaminase (ADA) activity in the small intestine of mice. ADA activity was measured after administering indicated doses (A) of Bt_2-cAMP and at indicated time intervals (B). Other experimental details are mentioned in Materials and Methods section. Values are means for 4–5 mice. Bars, S.D. Asterisks represent statistically significant ($P < 0.01$) values as compared to control.

survival. Both B and T lymphocytes are extremely sensitive to adenosine and deoxyadenosine. Several reports have suggested that the secondary lymphoid organ such as intestinal epithelium can serve as a T-cell generative organ and might influence T-cell receptor (TCR) selection [16]. Adenosine exerts a wide range of biological effects by interacting with specific cell-surface receptor coupled to adenylate cyclase system. These receptors (A_1/A_2) regulate adenylate cyclase either by inhibiting or stimulating, thereby influencing the intracellular level of cAMP [17].

The normal endogenous level of intestinal ADA activity is very low at day 5, which reaches a peak value at day 20 of postnatal age. Higher level of ADA may ensure lower adenosine and better survival of lymphoid cells and hence, favour local immune responses at the mucosal surface of gastrointestinal tract during weaning period of mice. Alternatively, elevated level of ADA may also function to ensure that dietary sources of adenosine do not exert unwanted physiological effects during weaning [10]. The decrease in ADA activity at day 30 and 60 reflect functions, other than those attributed during weaning period. Our findings differ from earlier report of Chinsky *et al.* [9], wherein no marked decrease was observed in ADA activity of proximal intestinal tube (mainly the duodenum). Whereas, our study included whole portion of small intestine comprising of duodenum, jejunum and ileum. We have also reported an elevation of ADA activity in the spleen and stomach of mice until 60-day of postnatal age [18] in contrast to intestinal ADA, where the activity drops during the same period.

Our findings of corticosterone inhibition of ADA activity can be correlated with the greater accumulation of adenosine and deoxyadenosine, that might produce lymphotoxicity and suppress immune responses [2]. Corticosterone has already been reported by our group to inhibit ADA activity in the spleen, stomach and liver tissues of mice [18]. Albeit, the magnitude of inhibition is

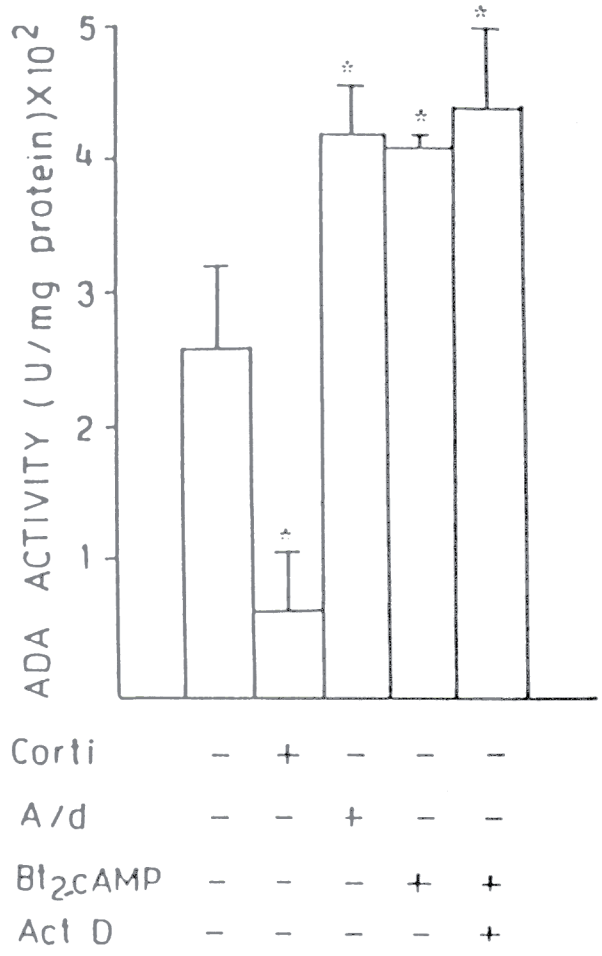

FIGURE 4 Effects of corticosterone (corti), adrenalectomy (A/d), dibutyryl-cAMP (Bt_2-cAMP) and actinomycin D (Act D) on the activity of adenosine deaminase (ADA) in the small intestine of mice. The doses of hormones and the adrenalectomy procedures are described in the Materials and Methods section. Activity of ADA was measured in the presence (+) or absence (−) of the indicated treatments. Values are means for 4–5 mice with the bars as standard deviation. Asterisks represent statistically significant ($P < 0.001$) values as compared to control.

less pronounced at early ages (15- and 30-day) as compared to the later age (60-day). This may be because of the better adaptive role and maturation of corticosterone action mechanism that includes its receptors and post-receptor events at 60-day of postnatal age [19]. We also confirmed our observation on corticosterone inhibition of ADA activity using adrenalectomized mice, wherein adrenalectomy caused significant increase in ADA activity. This clearly indicates that ADA is possibly under tonic inhibition by corticosterone. Our finding of corticosterone inhibition of ADA activity corroborates with the earlier report on suppression of ADA activity and its gene expression by dexamethasone, a synthetic glucocorticoid, in human leukemic cells [20].

We also observed that Bt_2-cAMP induces ADA activity in the small intestine of mice at all the ages studied. Bt_2-cAMP is an analog of cAMP, a well known second messenger for various proteins and peptide hormones. The intracellular level of cAMP is also influenced by ADA

substrate, adenosine. Depending on the concentration, adenosine binds either to A_1 or A_2 type receptors on cell surface and influence the level of cAMP [17]. Corroborating with the recent findings on the role of cAMP as an essential factor in immune responses [12], it may be suggested that cAMP might potentiate the immune response by stimulating ADA activity thereby lowering the level of adenosine to help better survival of lymphocytes. In order to assess the mode of induction of intestinal ADA by Bt_2-cAMP, we used actinomycin D whose prior administration did not show any effect on the ADA activity enhancement by Bt_2-cAMP. It indicates that the increase in ADA activity by Bt_2-cAMP may not be at the transcriptional level. However, the possibility of post-transcriptional and/or translational level regulation cannot be ruled out. A precise use of protein kinase inhibitors may be useful in understanding Bt_2-cAMP-dependent increase in ADA activity.

In conclusion, our findings indicate an age-specific expression of ADA in the small intestine of mice during postnatal development. We also found corticosterone as an inhibitory and Bt_2-cAMP as a stimulatory signal in controlling the ADA activity. These signals might be responsible for maintaining the endogenous level of ADA and could be useful in the management of ADA-related disorders.

Acknowledgements

We are grateful to the Department of Science and Technology, New Delhi, and the North Eastern Hill University, Shillong, for providing research facilities. L.S.S thanks the University for Senior Research Fellowship.

References

[1] Wilson, D. K., Quiocho, F. A. A pre-transition state mimic of an enzyme: X-ray structure of adenosine deaminase with bound 1-deazaadenosine and zinc-activated water. *Biochemistry* 1993; **32**: 1689–1694.

[2] Geiger, J. D., Padua, A. R., Nagy, J. I. Adenosine deaminase regulation of purine actions. In: Phillis J. W., ed. *Adenosine and Adenine Nucleotides as Regulators of Cellular Function*. CRC press: Boca Raton Florida, 1991: 71–84.

[3] Valerio, D., Duyvesteyn, M. G. C., Dekker, B. M. M., Weeda, G., Berkvens, Th. M., Vander Boorn, L., Van Ormondt, H., Van der El, A. J. Adenosine deaminase: characterization and expression of a gene with a remarkable promoter. *EMBO J.* 1985; **4**: 437–443.

[4] Hoang, I., Bergeoron, M. Adenosine deaminase in renal ontogeny and compensatory hypertrophy in the rat. *Cell Tissue Kinet.* 1983; **16**: 59–64.

[5] Dusing, M. R., Wiginton, D. A. Sp 1 is essential for both enhancer-mediated and basal activation of the TATA-less human ADA promoter. *Nucleic Acids Res.* 1994; **22**: 669–677.

[6] Berkvens, T. M., Schoute, F., Van Ormondt, H., Merra Khan, P., Van der Eb, A. J. Adenosine deaminase gene expression is regulated posttranscriptionally in the nucleus. *Nucleic Acids Res.* 1988; **16**: 3255–3262.

[7] Hershfield, M. S., Chaffee, S., Sorensen, R. U. Enzyme replacement therapy with polyethylene glycol-adenosine deaminase in adenosine deaminase deficiency: overview and case reports of three patients, including two now receiving gene therapy. *Pediat. Res.* 1993; **33**: S42–S48.

[8] Valentine, W. M., Pablia, D. E., Tartaglia, A. P., Gilsanz, F. Hereditary hemolytic anemia with increased red cell adenosine deaminase (45- to 70-fold) and decreased adenosine triphosphate. *Science* 1977; **195**, 783–784.

[9] Chinsky, J. M., Ramamurthy, V., Fanslow, W. C., Ingolia, D. E., Blackburn, M. R., Shaffer, K. T., Higley, H. R., Trentin, J. J., Rudolph, F. B., Knudsen, T. B., Kellems, R. E. Developmental expression of adenosine deaminase in the upper alimentary tract of mice. *Differentiation* 1990; **42**: 172–183.

[10] Mohamedali, K. A., Guicherit, O. M., Kellems, R. E., Rudolph, F. B. The highest levels of purine catabolic enzymes in mice are present in the proximal small intestine. *J. Biol. Chem.* 1993; **268**: 23728–23733.

[11] Strohmeier, G. R., Reppert, S. M., Lencer, W. I., Madara, J. L. The A2b adenosine receptor mediates cAMP responses to adenosine receptor agonists in human intestinal epithelia. *J. Biol. Chem.* 1995; **270**: 2387–2394.

[12] Koh, W. S., Yang, K. H., Kaminski, N. E. Cyclic AMP is an essential factor in immune responses. *Biochem. Biophys. Res. Commun.* 1995; **206**: 703–709.

[13] Kalchar, H. M. Differential spectrophotometry of purine compounds by means of specific enzymes. III. Studies of the enzymes of purine metabolism. *J. Biol. Chem.* 1947; **167**: 461–475.

[14] Yoshida, S. Y., Aikawa, T. Partial characterization of the adenosine deaminase purified from the scallop midgut gland. *Comp. Biochem. Physiol.* 1993; **105B**: 51–61.

[15] Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Anal. Biochem.* 1976; **72**: 248–254.

[16] Poussier, P., Edouard, P., Lee, C., Binnie, M., Julius, M. Thymus-independent development and negative selection of T-cells expressing T-cell receptor α/β in the intestinal epithelium: evidence for distinct circulation patterns of gut and thymus-derived T lymphocytes. *J. Exp. Med.* 1992; **176**: 187–200.

[17] Dalziel, H. H., Westfall, D. P. Receptors for adenine nucleotides and nucleosides: subclassification, distribution and molecular characterization. *Pharmacol. Rev.* 1994; **46**: 449–466.

[18] Singh, L. S., Sharma, R. Developmental expression and corticosterone inhibition of adenosine deaminase activity in different tissues of mice. *Mech. Ageing and Dev.* 1995; **80**: 85–92.

[19] Ming-Jer, T., O'Malley, W. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Ann. Rev. Biochem.* 1994; **63**: 451–486.

[20] Fernandez-Mejia, C., Peralta-Zaragoza, O., Cerezo-Roman, J., Navarro-Duque, C., Barrena-Rodriguez, R., Martinez-Valdez, H., Madrid-Marina, V. Regulation of gene expression of adenosine dearninase, purine nucleoside phosphorylase and terminal deoxynucleotidyl transferase by dexamethasone and cAMP in human leukemic cells. *Adv. Exp. Med. Biol.* 1994; **370**: 249–252.