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Antithetical effects of corticosterone and dibutyryl cAMP on adenosine deaminase in the gastrointestinal tract of chicken during postnatal development

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Abstract Adenosine deaminase (ADA; EC 3.5.4.4) is a purine catabolic enzyme causing hydrolytic deamination of adenosine and 2′-deoxyadenosine to inosine and 2′-deoxyinosine. In the present study, the normal endogenous activity level of ADA was investigated in different regions of the gastrointestinal tract (GIT) during postnatal development of chicken. The effects of corticosterone and dibutyryl cAMP (Bt_2-cAMP) were studied at two selected postnatal ages. The results indicated a significantly high level of ADA at day 1 in all the regions of GIT, which then declined (−34% in esophagus, −35% in crop, and −48% in small intestine) at day 10 and remained fairly constant till day 90. While in the proventiculus, the activity of ADA decreased (−30%) at day 30 and showed further decline (−52%) at day 90 as compared to day 1. Corticosterone was seen to significantly decrease (−23 to 79%) the activity level, depending on the regions of GIT studied except proventriculus. The magnitude of decline was more pronounced at day 60 compared to day 10. Bt_2-cAMP, on the other hand, caused a significant increase (+21 to 67%) in the activity level of ADA again depending on the regions of GIT studied except crop. Western blot analyses also depicted that the decrease and/or increase, respectively, of ADA activity by corticosterone and Bt_2-cAMP was at the ADA protein level. In conclusion, the study suggests that the ADA activity level is highest at day 1 in all the regions of chicken GIT and could be reduced or enhanced by corticosterone and dibutyryl cAMP, respectively, in an age-specific manner.

Keywords Adenosine deaminase · Corticosterone · Bt_2-cAMP · GIT and chicken

Introduction

The purine salvage pathway enzyme adenosine deaminase (ADA; EC 3.5.4.4) catalyses the irreversible breakdown of adenosine and 2′-deoxyadenosine to inosine and 2′-deoxyinosine, respectively [1, 2]. The activity of ADA in lymphocytes and lymphoid organs has been considered as an index of immune response [3], since its deficiency is associated with impaired normal immune function [4] and combined immunodeficiency [5]. In aves, as in mammals, growth and development are controlled by interplay of various cellular signaling with the genes and their products. A number of hormones and growth factors exert an intricate, but judicious control on the process of development in aves. Not much is known about the role of chicken ADA as a function of postnatal development. During development of chicken, studies on the activity of ADA in B and T cells revealed a higher expression in the B lymphocytes. Thus, it plays a pivotal role in the maturation of the immune system in chicken [6]. ADA is a well-characterized enzyme involved in the depletion of adenosine levels. Adenosine, a local hormone, produced by mesangial cells is also a metabolic regulator of renal blood flow, capable of decreasing glomerular filtration rate (GFR), exerting immunosuppressive, antiproliferative, and anti-inflammatory properties [7]. It has also been shown that in chicken

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embryonic neurons, deficiency of ADA is associated with an accumulation of adenosine and generation of neurotoxicity [8, 9]. Signaling through extracellular adenosine receptors is believed to account for the wide-ranging effects of adenosine [10]. ADA metabolizes extracellular adenosine, so the deficiency of it results in an exacerbation of inflammation [11]. Hence, ADA is a crucial enzyme involved in the downregulation of the substrates, adenosine and 2′-deoxyadenosine. ADA has been reported to modulate cell growth in colon cancer cell lines [12, 13]. It was earlier shown that agents that trigger colon cancer cell line HT29 differentiation also trigger modifications in the activity of ADA [14]. A concomitant downregulation of adenosine A1 receptors and upregulation of adenosine A2 receptors occurred in cloned cells of colon cancer cell line with a strong reduction in proliferation [15]. Removal of exogenous adenosine by growth in the presence of adenosine deaminase also inhibited the proliferation of other human tumor cells [16].

In the present investigation, the normal endogenous level of ADA at different postnatal ages of chicken was studied in the GIT. The effects of corticosterone and Bt_2-cAMP on the activity of ADA were also studied. Corticosterone has various metabolic effects via binding to its cognate intracellular receptor and influencing the expression of those genes, which have specific cis-acting DNA sequences called GREs. It has earlier been reported from this laboratory that corticosterone inhibits ADA activity in mouse tissues [17]. It is known that cAMP is produced as an intracellular second messenger in response to a variety of protein/peptide hormonal signals. It regulates a wide range of important biological processes like cell metabolism, cell division, cell differentiation, growth, and neoplastic transformation [18]. Adenosine has been reported to partially inhibit drug-induced increase of cAMP in chicken liver [19]. Our findings indicate corticosterone inhibition and Bt_2-cAMP stimulation of ADA activity in GIT of chicken during postnatal ages of chicken, albeit to varying degrees.

Materials and methods

Animals

Male chicken (Babcock Venkateswara 380, BV 380 breed) were purchased locally from a veterinary farm. They were maintained at 25 ± 2°C under normal laboratory conditions and fed with a chick mash diet (Premier Hatchery Ltd.) and tap water ad libitum. Chicken of five postnatal ages (1, 10, 30, 60, and 90 days) were used to determine the normal endogenous level of ADA activity. For the hormonal studies, two postnatal ages (10 and 60 days) were selected for the experiments. Institutional guidelines were followed during experimentation.

Chemicals

A rabbit polyclonal antibody raised against amino acids 64–363 of human adenosine deaminase was obtained from Santa Cruz Biotechnology, Inc., USA. Goat-antirabbit-IgG-ALP conjugate was obtained from Bangalore Genei, India. Adenosine, corticosterone, and Bt_2-cAMP were purchased from Sigma Chemical CO., USA. All other chemicals used were of analytical grade. Glass distilled water was used in the preparation of buffers.

Buffers

These were as follows: (A) 0.25 M sucrose/100 mM sodium citrate, pH 5.5; (B) 100 mM sodium citrate, pH 5.5; (C) Tris buffer saline (TBS; 20 mM Tris–HCl, pH 7.5/50 mM NaCl); (D) Tween Tris buffer saline (TTBS; 20 mM Tris–HCl, pH 7.5/50 mM NaCl/0.5% Tween 20); (E) Phosphate buffer saline (PBS; 0.14 M NaCl/3 mM KCl/10 mM Na_2HPO_4/2 mM KH_2PO_4, pH 7.3); and (F) Towbin buffer (25 mM Tris/192 mM glycine/20% w/v methanol, pH 8.3).

Preparation and assay of ADA

Chicks were killed by decapitation at a fixed time of the day (15:00 h). The regions of GIT (esophagus, crop, proventriculus, and small intestine) were dissected out, washed in normal saline (0.9% NaCl), and blotted dry. A 20% (w/v) homogenate was prepared in chilled buffer A. The homogenates were centrifuged at 20,000 × *g* for 45 min at 4°C to obtain a clear fat-free cytosol. The supernatant thus obtained was used for the assay of the enzyme. The activity of ADA was measured spectrophotometrically in a Hitachi Model-U2000 spectrophotometer using the method of Kalckar [20] and Yoshida and Aikawa [21] with certain modifications of our own research [17]. The 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 ml of buffer B containing 100 μM adenosine and 50 μl of suitably diluted enzyme preparation, which gave a linear decrease in absorbance at 265 nm.

Hormonal treatment

Corticosterone injection

Pilot experiments were undertaken to determine the time- and dose–response of corticosterone in influencing ADA activity [17]. Finally, corticosterone was administered at a

dose of 1.0 mg/100 g body weight in 0.3 ml normal saline having 6% ethanol intraperitoneally. Control animals received an equal amount of saline and ethanol solution. All the chicks were killed after 6 h of treatment.

Bt_2-cAMP injection

Similarly, the time– and dose–response of Bt_2-cAMP, a membrane permeable analog of cAMP, was ascertained for regulating ADA activity. Finally, Bt_2-cAMP was administered at a dose of 1.0 mg/100 g body weight in 0.3 ml normal saline, intraperitoneally. Control chicks received an equal amount of saline only. The controls and treated chicken were killed after 4 h of injection.

Western blot analysis

An aliquot of cytosol (30 μl) containing 50 μg protein was loaded in each lane. After the protein samples were subjected to SDS-PAGE (8%), the gels were taken out and incubated in buffer F for 10–15 min for equilibration. The transfer was carried out in a chilled buffer F using Bio-Rad Mini trans-blot® electrophoretic transfer cell at 100 V (constant) for 60 min. Bio Ice cooling unit was also used to maintain appropriate buffer temperature. The electroblotted nitrocellulose membranes were then processed for immunodetection. The membrane was transferred in blocking solution (5% nonfat milk in buffer C). It was washed twice in buffer D with gentle agitation and incubated overnight with anti-ADA antibody solution (1:500). Using BLASTP 2.2.19+, the homology between human (acc. no. NP000013) and chicken (acc. no. Q5ZKP6) ADA was found to be 66% [22]. The cross-reactivity of the antibody used to chicken ADA was tested to be ~40% by immunotitration. The membrane after overnight incubation was then washed twice in buffer D to remove unbound antibodies. Then after, the membrane was transferred to a solution containing goat-antirabbit-IgG-ALP conjugate (1:10000). This was allowed to incubate for 3 h. The membrane was washed twice in buffer D and finally washed in buffer C to remove the detergent. For the development of color, the substrate (BCIP/NBT) was added and the membrane kept at 37°C till the development of color. The reaction was stopped by washing the membrane in ddH_2O and photographed using an hp scanjet 7400 c scanner.

India ink stain

In order to ascertain equal amount of loads in each slot, a parallel set was run and stained overnight with 0.2% India ink in Tween solution (0.3% Tween 20 in buffer E). Destaining was performed with Tween solution. The blot was photographed using an hp scanjet 7400 c scanner.

Protein estimation

Protein content of the enzyme preparation was measured according to the dye-binding method of Bradford [23] using bovine serum albumin (BSA) as standard.

Statistical analysis

Data obtained from different sets of experiments were analyzed statistically. The results were expressed as mean ± SD. Statistical evaluation of the data was made by means of Student's *t*-test for paired data. Comparisons were considered significant at $P < 0.001$.

Results

Normal endogenous level of ADA activity

Among the regions of GIT, the highest level of ADA activity was found in the small intestine, followed by the esophagus, proventriculus, and crop (Fig. 1a–d). The specific activity of ADA in all the regions of the GIT exhibited a high level at day 1. At day 10, a sharp decline (−34% in esophagus, −35% in crop, and −48% in small intestine) in the activity of ADA was seen in all regions of GIT, except proventiculus, where the activity of ADA decreased (−30%) at day 30 and showed further decline (−52%) at day 90 as compared to day 1. While in other regions, the activity of ADA remained fairly constant after day 10. Western blot analysis of ADA protein in the selected postnatal ages, days 1 and 10 indicated that the decline was at the ADA protein level (Fig. 2i). India ink stain indicated that an equal amount of protein was loaded in each slot (Fig. 2ii) during experimentation taking a major GIT protein of ~42 kDa as a reference. Densitometric analysis of Western blots using Kodak Digital Science (KDS-1) software indicated a similar pattern (data not shown).

Effect of corticosterone on ADA activity

The effect of corticosterone on ADA activity was studied in the GIT of chicken at two selected postnatal ages (10 and 60 days old), wherein the level of ADA was fairly similar. Our studies showed that corticosterone significantly inhibited the ADA activity in all the regions studied except proventriculus (Fig. 5a), in an age- and region-specific manner. The activity of ADA was significantly inhibited by corticosterone in the esophagus, but more at day 60 (−41%; $P < 0.001$) in comparison with day 10 (−23%; $P < 0.001$) (Fig. 3a). In the crop, corticosterone inhibited the activity at both ages, but the magnitude of inhibition was more pronounced in day 60 (−79%; $P < 0.001$) than

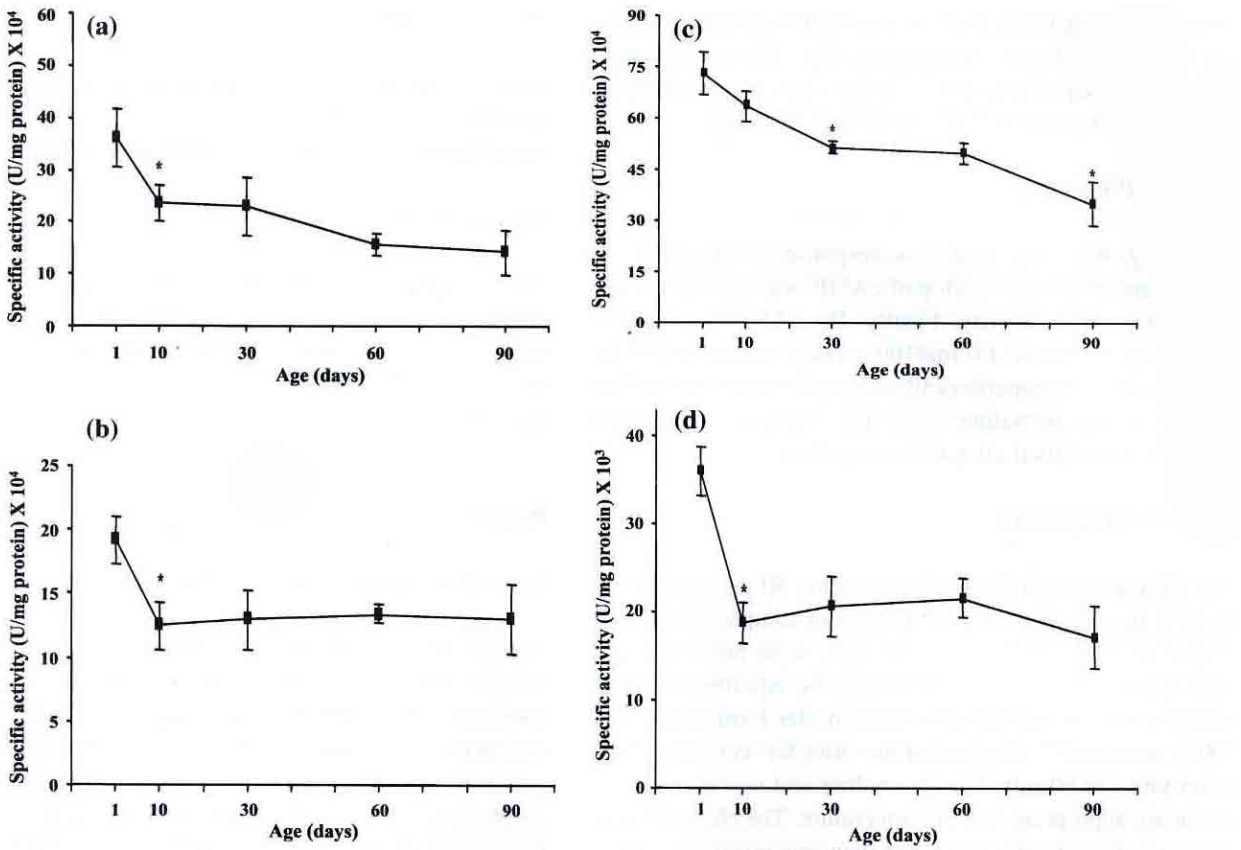

Fig. 1 Normal endogenous level of adenosine deaminase activity in different regions of GIT of chicken at various postnatal ages: esophagus (**a**), crop (**b**), proventriculus (**c**), and small intestine (**d**). Values are expressed as mean from five chicken at each point. *Bars* represent standard deviation. *Asterisks* (*) indicates statistically significant ($P < 0.001$) value as compared to day 1

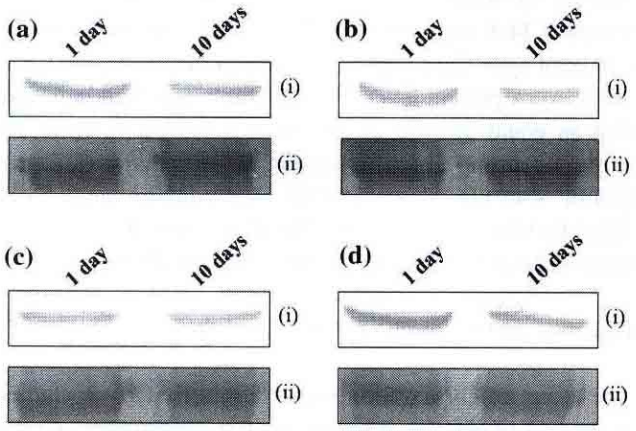

Fig. 2 (i) Western blot analysis of ADA from esophagus (**a**), crop (**b**), proventriculus (**c**), and small intestine (**d**) of chicken from days 1 to 10. An equal amount (50 μg protein) of cytosol containing ADA was loaded in each lane and processed for immunoblotting using human polyclonal ADA antibody. (ii) The India ink stained Western blots for a major GIT protein of ~42 kDa on nitrocellulose membrane

at day 10 (−56%; $P < 0.001$) (Fig. 4a). In small intestine, the percent inhibition of the activity was seen to be more at day 60 (−41%; $P < 0.001$) than at day 10 (−36%; $P < 0.001$) (Fig. 6a). Western blot analysis as done above corroborated the decrease in ADA activity at the protein expression level [Figs. 3–6b(i)]. India ink stain indicated that an equal amount of protein was loaded in each slot [Figs. 3–6b(ii)].

Effect of Bt_2-cAMP on ADA activity

In search of a stimulatory regulator of ADA activity, the effect of Bt_2-cAMP, a membrane permeable analog of cAMP was studied on the activity of ADA in the GIT of chicken at two selected postnatal ages as followed for corticosterone treatment. In the esophagus, Bt_2-cAMP enhanced the ADA activity more in day 60 (+67%; $P < 0.001$) than in day 10 (+42%; $P < 0.001$) (Fig. 3a). In the crop, the activity of ADA was not influenced by Bt_2-cAMP at either age (Fig. 4a). However, the level of activity of ADA in proventriculus was almost similarly enhanced (+21%; $P < 0.001$ and +29%; $P < 0.001$) in both the ages (Fig. 5a). In the small intestine, the percent induction of activity was seen to be more at day 60 (+62%; $P < 0.001$) than at day 10 (+51%; $P < 0.001$) (Fig. 6a). Western blot analysis supported the observation that the increase in ADA activity by Bt_2-cAMP was at the protein level

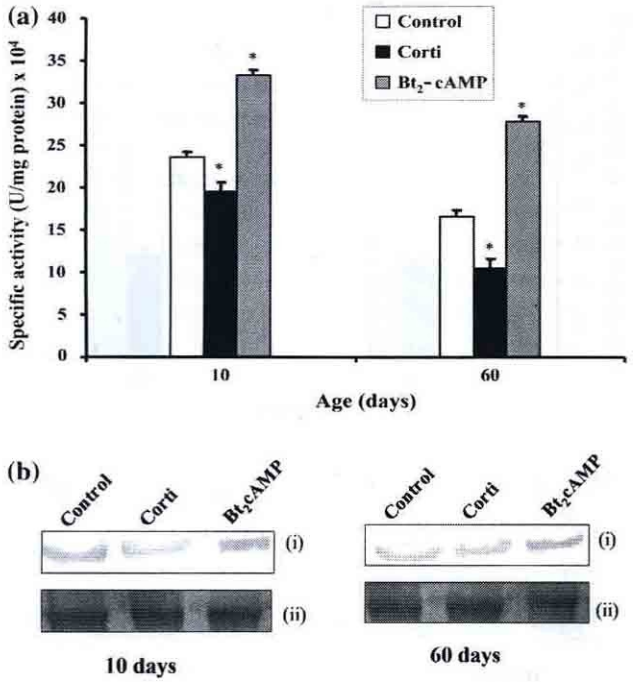

Fig. 3 **a** Effect of corticosterone (Corti) and Bt_2-cAMP on the activity of ADA in esophagus at two postnatal ages (days 10 and 60). Values are expressed as mean from five chicken in each group. *Bars* represent standard deviation. *Asterisks* (*) indicate statistically significant values as compared to control ($P < 0.001$). **b** (i) Western blot analysis of ADA from esophagus. An equal amount (50 μg protein) of cytosol containing ADA was loaded in each lane and processed for immunoblotting using human polyclonal ADA. (ii) The India ink stained Western blots for a major GIT protein of ~42 kDa on nitrocellulose membrane

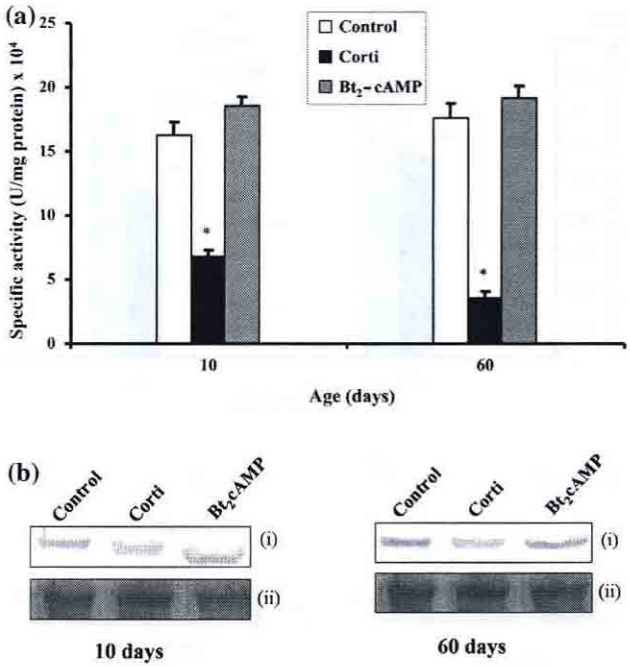

Fig. 4 **a** Effect of corticosterone (Corti) and Bt_2-cAMP on the activity of ADA in crop at two postnatal ages (days 10 and 60). Values are expressed as mean from five chicken in each group. *Bars* represent standard deviation. *Asterisks* (*) indicate statistically significant values as compared to control ($P < 0.001$). **b** (i) Western blot analysis of ADA from crop. An equal amount (50 μg protein) of cytosol containing ADA was loaded in each well and processed for immunoblotting using human polyclonal ADA. (ii) The India ink stained Western blots for a major GIT protein of ~42 kDa on nitrocellulose membrane

[Figs. 3–6b(i)]. India ink staining suggested that an equal amount of protein was loaded in each slot [Figs. 3–6b(ii)].

Discussion

The levels of adenosine and 2′-deoxyadenosine are controlled by adenosine deaminase that catalyzes the irreversible breakdown of these metabolites [24]. The physiologic consequences of these metabolites are well documented in individuals genetically deficient in this enzyme [25]. In normal individuals, ADA appears to be present in high amounts in lymphoid tissues to maintain low concentrations of adenosine and deoxyadenosine permitting better lymphocyte survival. Both B and T lymphocytes are extremely sensitive to adenosine and deoxyadenosine [26]. Elevated levels of deoxyadenosine often lead to cytotoxicity resulting from interference with deoxynucleotide metabolism [25]. Adenosine functions as an extracellular signal transducer mediating a vast array of biological effects by interacting with specific cell surface receptors coupled to adenylate cyclase system. These receptors (A1/A2) regulate adenylate cyclase by inhibiting or stimulating and influencing the intracellular level of cAMP, respectively.

Our findings reveal that the normal endogenous level of ADA in the GIT of chicken is highest on the day of hatching. Among the regions of the GIT studied, the highest level of ADA activity is found in the small intestine followed by the esophagus, proventriculus, and crop. In the esophagus, the activity is highest in day 1 and is seen to decrease at day 10 and shows a gradual decrease thereafter. In the crop, ADA activity is highest in day 1 showing a decrease at day 10 and then no decline in the activity. The proventriculus is also seen to have a very high level of ADA activity at day 1, which shows a significant decline at days 30 and 90. Region-specific studies indicated that in the small intestine, the level of activity is highest in day 1 followed by a sharp decrease at day 10 and then remain almost constant thereafter. The avian gastrointestinal mucosal immune system has evolved with specialized features that reflect their role as the first line of defense on mucosal surfaces [27]. High level of ADA on the day of hatching may ensure lower adenosine and better survival of lymphoid cells. The presence of high level of ADA activity in the upper alimentary canal probably serves a catabolic

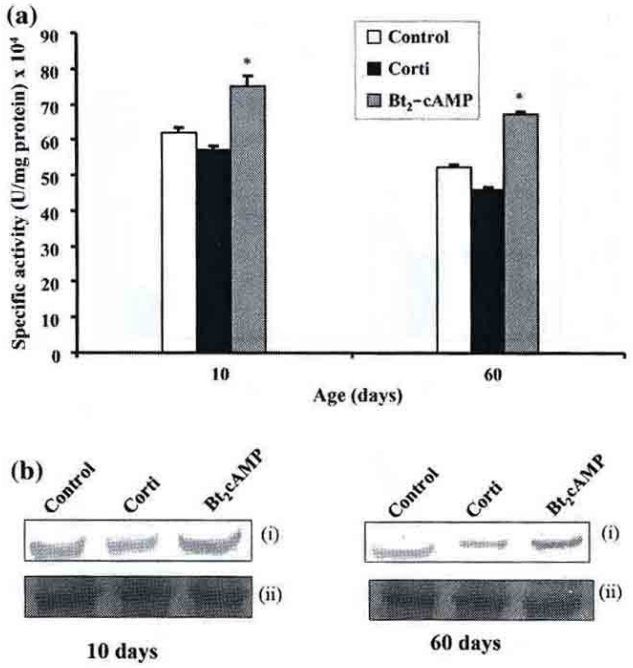

Fig. 5 **a** Effect of corticosterone (Corti) and Bt_2-cAMP on the activity of ADA in proventriculus at two postnatal ages (days 10 and 60). Values are expressed as mean from five chicken in each group. *Bars* represent standard deviation. *Asterisks* (*) indicate statistically significant values as compared to control ($P < 0.001$). **b** (i) Western blot analysis of ADA from proventriculus. An equal amount (50 μg protein) of cytosol containing ADA was loaded in each well and processed for immunoblotting using human polyclonal ADA. (ii) The India ink stained Western blots for a major GIT protein of ~42 kDa on nitrocellulose membrane

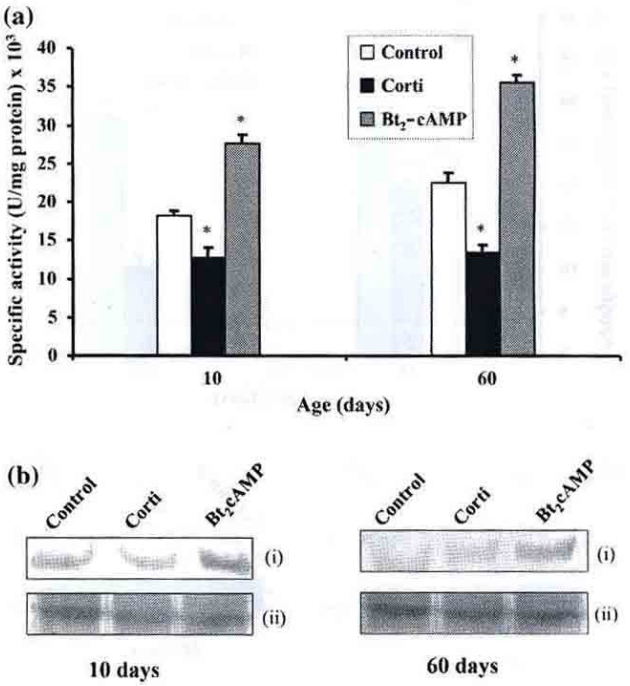

Fig. 6 **a** Effect of corticosterone (Corti) and Bt_2-cAMP on the activity of ADA in small intestine at two postnatal ages (days 10 and 60). Values are expressed as mean from five chicken in each group. *Bars* represent standard deviation. *Asterisks* (*) indicate statistically significant values as compared to control ($P < 0.001$). **b** (i) Western blot analysis of ADA from small intestine. An equal amount (50 μg protein) of cytosol containing ADA was loaded in each well and processed for immunoblotting using human polyclonal ADA. (ii) The India ink stained Western blots for a major GIT protein of ~42 kDa on nitrocellulose membrane

role to guard the chicken against dietary sources of adenosine, which could exert unwanted physiologic effects. The newly hatched chicken is thus well disposed to face the onslaught of high sources of adenosine which accumulate during development. As adenosine and 2′-deoxyadenosine are lymphotoxic, highest levels of ADA in the small intestine may maintain low cellular concentration of these metabolites and assist a better survival milieu for lymphocytes. The Western blot analysis of ADA protein at two selected ages of days 1 and 10 corroborate the activity decline at day 10 of ADA compared to day 1 except proventriculus. Such findings clearly show that the higher activity of ADA level at day 1 is because of its protein level. It has earlier been reported that newly hatched chicken showed a significantly higher ADA activity in bursa tissue regardless of whether ADA activity is expressed per milligram of wet tissue, per 10^9 cells or per milligram tissue or cell protein [28].

Our studies also showed that corticosterone significantly inhibited the ADA activity in all the regions of GIT except proventriculus, in an age- and region-specific manner. In these regions of GIT, the magnitude of inhibition was more pronounced at the later stage (day 60) of development in comparison to the younger age (day 10). Corticosterone inhibition of ADA activity may be correlated with the greater accumulation of adenosine and 2′-deoxyadenosine, which cause lymphotoxicity, suppressing immune responses [18, 29]. ADA converts adenosine to nontoxic inosine, protecting lymphoid cells against the cytotoxic effects of adenosine [30]. The findings of age- and tissue-specific inhibition may be correlated with the differential adaptive role and maturation of corticosterone action mechanism, its receptor and postreceptor events [31, 32]. Pronounced inhibition at a later stage may be attributed to greater maturation of corticosterone receptors, thus facilitating greater binding of the hormone to its receptor. Corticosterone is known to act by binding to specific intracellular receptors and by interaction of these hormone–receptor complexes to specific DNA sequences, called hormone responsive elements (HREs), thereby modulating the transcription of specific gene(s) [33, 34]. Inhibition of ADA activity level may result in significant attenuation of intestinal inflammation by corticosterone [35], justifying its anti-inflammatory role other than the established mode via inhibiting production of inflammatory agents.

In search of a positive modulator, Bt_2-cAMP was found to increase the activity of ADA in all regions of GIT studied except crop in an age- and region-specific manner. In the GIT, the activity level of ADA was found to be greatly enhanced at a later age (day 60) of chicken development when compared to the young age (day 10). This may be because of the differential expression of secondary messenger cascade at the later stage of GIT development. Bt_2-cAMP is a well-known second messenger for various protein and peptide hormones [36, 37]. The substrate adenosine influences the intracellular concentration of cAMP [38]. The findings reveal that adenosine may control its own intracellular level through cAMP that may induce the activity of ADA, which might lower the increased level of adenosine and avoid the unwanted toxic effects of adenosine [1]. The immunoinducing role of cAMP might be because of stimulating activity of ADA, thereby decreasing the intracellular concentration of adenosine, ensuring a better environment for lymphocyte proliferation.

Such induction and inhibition of ADA activity level by Bt_2-cAMP and corticosterone, respectively, was also ascertained using Western blot analysis that confirmed the induction and inhibition of ADA activity at ADA protein level. Thus, our studies conclude that the level of ADA is highest on the day of hatching and that the level is negatively and positively controlled by corticosterone and Bt_2-cAMP in various regions of GIT, albeit more pronounced at later postnatal ages, depending on the maturation of steroidal and protein/peptide hormone signaling cascade during postnatal development of chicken for better metabolic adjustments in growing animals. In addition, such regulators could be of use in treating a number of ADA-related diseases wherein the activity of ADA gets altered. It is concluded from the present findings that the ADA activity level is highest at day 1 in all the regions of chicken GIT and could be reduced or enhanced by corticosterone and dibutyryl cAMP, respectively, in an age-specific manner.

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References

1. Mohamedali KA, Guicherit OM, Kellems RE, Rudolf FP (1993) The highest levels of purine catabolic enzymes in mice are present in the proximal small intestine. J Biol Chem 268:23728–23733
2. Wilson DK, Quiocho FA (1993) A pre-transition state mimic of an enzyme: X-ray structure of adenosine deaminase with bound 1-deazaadenosine and zinc-activated water. Biochemistry 32: 1689–1694. doi:10.1021/bi00058a001
3. Hall JG (1963) Adenosine deaminase activity in lymphoid cells during antibody production. Aust J Exp Biol Med Sci 41:93–97. doi:10.1038/icb.1963.8
4. Daddona PE, Mitchell B, Menwisson JH, Devdson LB, Wilson MJ, Koller AC (1983) Adenosine deaminase deficiency with normal immune function. An acidic enzyme mutation. J Clin Invest 72:483–490. doi:10.1172/JCI110996
5. Donofrio J, Coleman MS, Hulton JJ (1978) Overproduction of adenine deoxynucleosides and deoxynucleotides in adenosine deaminase deficiency with severe combined immunodeficiency disease. J Clin Invest 62(4):884–887. doi:10.1172/JCI109201
6. Senesi S, Batoni G, Bianchi F, Freer G, Dolfi A, Campa M, Lupetti M (1990) Questioning the role of adenosine deaminase in development of B-lymphocytes in chicken bursa. Dev Comp Immunol 14(1):95–104. doi:10.1016/0145-305X(90)90011-3
7. Kocic G, Djordjevic V, Vlahovic P, Kocic R, Pavlovic D, Jevtovic T (2002) Antioxidants modulate adenosine metabolism in rat mesangial cells cultured under high glucose conditions. Ren Fail 24(6):691–701. doi:10.1081/JDI-120015665
8. Wakade AR, Przywara DA, Palmer KC, Kulkarni JS, Wakade TD (1995) Deoxynucleoside induces neuronal apoptosis independent of neurotrophic factors. J Biol Chem 270(30):17986–17992. doi:10.1074/jbc.270.30.17986
9. Zhao Z, Crossland WJ, Kulkarni JS, Wakade TD, Wakade AR (1999) 2′-Deoxyadenosine causes cell death in embryonic chicken sympathetic ganglia and brain. Cell Tissue Res 296(2): 281–291. doi:10.1007/s004410051289
10. Gomez G, Apasov S, Sitkovsky MV (2001) Immunosuppressive effects of extracellular adenosine on immune cells: implications for the pathogenesis of ADA SCID and immunomodulation. Drug Dev Res 53:218–224. doi:10.1002/ddr.1190
11. Kuno M, Seki N, Tsujimoto S, Nakanishi I, Kinoshita T, Nakamura K, Terasaka T, Nishio N, Sato A, Fujii T (2006) Anti-inflammatory activity of non-nucleoside adenosine deaminase inhibitor FR234938. Eur J Pharmacol 534(1–3):241–249. doi:10.1016/j.ejphar.2006.01.042
12. Lelievre V, Muller JM, Falcon J (1998) Adenosine modulates cell proliferation in human colonic carcinoma. II. Differential behavior of HT29, DLD-1, Caco-2 and SW403 cell lines. Eur J Pharmacol 341:299–308. doi:10.1016/S0014-2999(97)01463-5
13. Lelievre V, Muller JM, Falcon J (1998) Adenosine modulates cell proliferation in human colonic adenocarcinoma. I. Possible involvement of adenosine A1 receptor subtypes in HT29 cells. Eur J Pharmacol 341:289–297. doi:10.1016/S0014-2999(97)01462-3
14. Dexter DL, Crabtree GW, Stoeckler JD, Savarese TM, Ghoda LY, Rogler-Brown TL, Parks RE Jr, Calabresi P (1981) *N,N*-Dimethylformamide and sodium butyrate modulation of the activities of purine-metabolizing enzymes in cultured human colon carcinoma cells. Cancer Res 41(3):808–812
15. Lelievre V, Caigneaux E, Muller JM, Falcon J (2000) Extracellular adenosine deprivation induces epithelial differentiation of HT29 cells: evidence for a concomitant adenosine A(1)/A(2) receptor balance regulation. Eur J Pharmacol 391:21–29. doi:10.1016/S0014-2999(00)00048-0
16. Colquhoun A, Newsholme EA (1997) Inhibition of human tumour cell proliferation by analogues of adenosine. Cell Biochem Funct 15(2):135–139. doi:10.1002/(SICI)1099-0844(199706 01)15:2<135::AID-CBF733>3.0.CO;2-G
17. Singh LS, Sharma R (1995) Developmental expression and corticosterone inhibition of adenosine deaminase activity in different tissues of mice. Mech Ageing Dev 80(2):85–92. doi:10.1016/0047-6374(94)01560-9
18. Mei FC, Qiao J, Tysgankova OM, Meinkoth JL, Quilliam LA, Cheng X (2002) Differential signaling of cAMP: opposing effects of exchange protein directly activated by cyclic AMP and cAMP-

dependent protein kinase on protein kinase B activation. J Biol Chem 277(13):11497–11504. doi:10.1074/jbc.M110856200
19. Bhat NR, Kulkarni GR, Rao MA, Murthy SK (1979) Studies on the inhibition of fatty acid synthesis in the chicken liver by adenine compounds in vitro. J Biosci 1(4):69–375. doi:10.1007/BF02704618
20. Kalckar HM (1947) Differential spectrophotometry of purine compounds by means of specific enzymes III. Studies of the enzymes of purine metabolism. J Biol Chem 167:461–475
21. Yoshida SY, Aikawa T (1993) Partial characterization of the adenosine deaminase purified from the scallop midgut gland. Comp Biochem Physiol B 105:51–61. doi:10.1016/0305-0491(93)90168-5
22. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402. doi:10.1093/nar/25.17.3389
23. Bradford MA (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye-binding. Anal Biochem 72:248–254. doi:10.1016/0003-2697(76)90527-3
24. Fox IH, Kelley WN (1978) The role of adenosine and 2′-deoxyadenosine in mammalian cells. Annu Rev Biochem 47:655–686. doi:10.1146/annurev.bi.47.070178.003255
25. Hershfield MS, Mitchell BS (1995) Immunodeficiency diseases caused by adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited diseases. McGraw-Hill, New York, pp 1725–1768
26. Pollok KE, Hanenberg H, Noblitt TW, Schroeder WL, Kato I, Emanuel D, Williams DA (1998) High-efficiency gene transfer into normal and adenosine deaminase-deficient T lymphocytes is mediated by transduction on recombinant fibronectin fragments. J Virol 72(6):4882–4892
27. Hyun S, Lilleho J, James M (1996) Trout avian gut-associated lymphoid tissues and intestinal immune responses to Eimeria parasites. Clin Microbiol Rev 9(3):349–360
28. Ratech H, McCarthy G, Meuwissen HJ (1980) Adenosine deaminase activity in lymphoid tissues of the developing chicken. Comp Biochem Physiol B 67(2):333–335. doi:10.1016/0305-0491(80)90153-4
29. Priebe TS, Nelson JA (1991) Adenosine and immune system function. In: Phillis JW (ed) Adenosine and adenine nucleotides as regulators of cellular function. CRC Press, Boca Raton, pp 141–167
30. Carson DA, Seegmiller JE (1976) Effect of adenosine deaminase inhibition upon human lymphocyte blastogenesis. J Clin Invest 57(2):274–282. doi:10.1172/JCI108278
31. Kalimi M, Hubbard J, Gupta S (1988) Modulation of glucocorticoid receptor from development to aging. Ann N Y Acad Sci 529:149–154. doi:10.1111/j.1749-6632.1988.tb35273.x
32. Borbhuiya MA, Sharma R (1995) Regulation of hepatic glucocorticoid receptor during development of mice. Biochem Mol Biol Int 37(4):645–652
33. Yamamoto KR (1985) Steroid receptor regulated transcription of specific genes and gene networks. Ann Rev Genet 19:209–252
34. Tsai M, O' Malley BW (1994) Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 63:451–486. doi:10.1146/annurev.bi.63.070194.002315
35. Antonioli L, Fornai M, Colucci R, Ghisu N, Da Settimo F, Natale G, Kastsiuchenka O, Duranti E, Virdis A, Vassalle C, La Motta C, Mugnaini L, Breschi MC, Blandizzi C, Del Taca M (2007) Inhibition of adenosine deaminase attenuates inflammation in experimental colitis. J Pharmacol Exp Ther 322(2):435–442. doi:10.1124/jpet.107.122762
36. Adashi EY, Resnick CE (1986) 3′,5′-Cyclic adenosine monophosphate as an intracellular second messenger of luteinizing hormone: application of the forskolin criteria. J Cell Biochem 31(3):17–228. doi:10.1002/jcb.240310304
37. Won HS, Lee J, Khil LY, Chae SH, Ahn MY, Lee BH, Chung JH, Kim YC, Moon CK (2004) Mechanism of action of brazilin on gluconeogenesis in isolated rat hepatocytes. Planta Med 70:740–744. doi:10.1055/s-2004-827205
38. Dalziel HH, Westfall DP (1994) Receptors for adenine nucleotides and nucleosides: subclassification, distribution, and molecular characterization. Pharmacol Rev 46:449–466