

## AGE- AND TISSUE- SPECIFIC REGULATION OF CHICKEN INORGANIC PYROPHOSPHATASE

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**Summary:** The activities and hormone regulatory patterns of inorganic pyrophosphatase (PPiase) of male Rhode Island Red (RIR) chickens were studied at two different postnatal ages (5- and 90-day). The endogenous activity level was found to be significantly higher in the immature (5-day) groups for all the tissues (liver, kidney and brain) studied as compared to that of mature (90-day). Hydrocortisone (HC) administration significantly inhibited the PPiase activity in the immature chicken liver and did not affect the enzyme in kidney and brain at either age. In contrast, insulin increased significantly the activity of PPiase in the liver of immature chicken. Kidney PPiase, however, was unaffected to insulin treatment at immature age, while it showed increased activity in mature group. On the other hand, brain PPiase activity was significantly increased at both the ages studied. These findings indicate an age- and tissue- specific regulation of PPiase activity by hydrocortisone and insulin in chicken.

**Keywords:** Chicken PPiase; Hydrocortisone; Insulin

### Introduction

Inorganic pyrophosphatase (EC 3.6.1.1; PPiase), is a ubiquitous enzyme that hydrolyses inorganic pyrophosphate (PPi) into two molecules of inorganic phosphate (Pi) with concomitant release of high energy. PPi is produced in a variety of cell metabolic processes such as, biosynthetic nucleoside triphosphate dependent reactions, like deoxyribo- and ribonucleotide polymerization, activation of amino-, fatty- acids and coenzyme synthesis (1-4). PPiase plays a dual role of replenishing the Pi pool for numerous metabolic reactions as well as carrying to completion the endergonic reactions in which PPi is formed (5). PPiase has been implicated in diverse functions such as bone metabolism (6) and in actively proliferating and/or growing tissues (7). Alterations in the level of enzymes and their inducibility by certain hormones are age-related phenomenon, characterized by changes in the responsiveness of tissues and cells to these hormones (8,9).

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Earlier work on PPIase has been done mostly on the mammalian systems (10-12). However, informations on the developmental and hormonal regulation of avian PPIase are scanty (13). The present work describes the endogenous activity level of male chicken PPIase at two ages, immature (5-day) and mature (90-day) in different tissues (liver, kidney and brain). In addition, the effects of hydrocortisone (HC) and insulin on the activity level of this enzyme is also reported and discussed for their regulatory roles.

### Materials and Methods

Animals and chemicals: Male chickens (Rhode Island Red, RIR breed) were locally purchased from a veterinary farm and maintained at  $25 \pm 2^\circ\text{C}$  with a 12 h light. Chickens were fed with a chick mash diet along with water *ad libitum*. Hydrocortisone and other biochemicals were from Sigma Chemical Co., USA. Insulin was from Boots Pharmaceutical, India.

Tissue preparation and assay of PPIase activity: Chickens of two postnatal ages (5-, and 90-day) were sacrificed at a fixed time of day (11:00) by decapitation. Tissues (liver, kidney and brain) were removed immediately, washed in ice-cold saline (0.9% NaCl) and blotted dry. A 10 % (w/v) homogenate of the tissues was prepared in ice-cold 0.25 M sucrose and centrifuged at  $27,500 \times g$  for 60 min at  $4^\circ\text{C}$ . The supernatants thus obtained were suitably diluted and used for inorganic pyrophosphatase assay.

PPIase was assayed according to the procedure of Weinhouse et al (14) incorporating some modifications (15). The assay procedure involves the catalytic hydrolysis of pyrophosphate into inorganic phosphate which complexes with the colour reagent (Malachite green-acid molybdate) to give a bluish-green colour that absorbs maximally at 660 nm. The intensity of colour is a direct measure of enzyme activity. Supernatants suitably diluted with buffer A [50 mM Tris-HCl buffer, pH 8.0/1 mM dithiothreitol (DTT)/1 mM  $\text{MgCl}_2$ ] were used for assay of PPIase. Enzyme preparation (10  $\mu\text{l}$ ) was added to 0.8 ml of buffer A and allowed to equilibrate at  $25^\circ\text{C}$  for 15 min in a water bath. The reaction was initiated by adding substrate (tetrasodium pyrophosphate prepared in buffer A) to a final concentration of 0.1 mM. The reaction was stopped after 15 min by transferring the tubes onto ice followed by addition of 0.1 ml of 2.4 M perchloric acid. Subsequently, 0.9 ml of phosphate colour reagent (0.1% malachite green/4.2% ammonium molybdate in 4 M HCl) was added to each tube, mixed properly and the colour developed was measured in a Hitachi U-2000 spectrophotometer. Routinely,  $\text{K}_2\text{HPO}_4$  reference standard as well as substrate, reagent and enzyme blanks were also run along with each assay. PPIase activity is expressed as  $\mu\text{mol}$  of orthophosphate formed/min/mg protein.

Protein Estimation: Protein concentrations were determined by the dye binding method of Bradford (16) using bovine serum albumin as reference standard.

Hormone treatments: Several trial experiments were undertaken to find out the time- and dose- response of the enzyme towards hydrocortisone and insulin. Maximum response of the enzyme for hydrocortisone was obtained with a repeated dose of 0.5 mg/100 g body weight for 3 days while insulin at a single dose (1U/100 g body weight) gave the optimal response. Hydrocortisone prepared in alcohol/saline mixture to a final alcohol strength of 10% was administered intraperitoneally (i.p.). Insulin was administered intramuscularly. Control chicks received equal amount of saline and/or ethanol/saline.

Blood sugar: To validate the effect of insulin, blood sugar was measured employing O'toluidine method (17). All the data were statistically analysed (18). The level of significance (p-values) between two sets of data was calculated according to student's t-test.

### Results and Discussion

Our results indicate that the normal endogenous activity of PPIase is significantly higher (20-30%) in the immature (5-day) chicken as compared to the mature (90-day) in all the tissues studied. Furthermore, the PPIase activity is highest in the liver followed by kidney and brain in both the age groups of chicken (Fig.1). The higher PPIase activity in the liver may entail the greater involvement of liver in biosynthetic processes compared to other tissues. Also the higher activity level of PPIase at early post-hatch period may contribute to increased rate of biosynthetic requirements of tissues at immature (5-day) as compared to mature (90-day) ages of chickens. A higher PPIase activity (40%) has also been reported in the red blood cells of newborns as compared to adults (11). An age-dependent decrease in the PPIase activity was also found in human aorta. However, in the rat liver, no age-dependent change in the activity of PPIase has been reported (10).

Hydrocortisone administration lowers (24%) the PPIase activity in the liver of immature chicks, while it does not influence the activity in brain and kidney at either of the ages studied. In contrast, insulin increases (54%) the activity of PPIase in the liver of 5-day old chicks and does not influence in 90-day old chickens (Fig.2A). However, it increases the PPIase activity in the kidney of 90-day old animals with no marked change at 5-day of postnatal age (Fig.2B). On the other hand, insulin increases significantly the activity of brain PPIase both in immature (35%) as well as in mature (22%) chicken (Fig. 2C). Blood sugar was monitored to ascertain the *in vivo* influence of exogenously administered insulin. Following insulin administration, serum level of glucose declined significantly in both immature (38%) and mature (30%) age-groups (Table I)

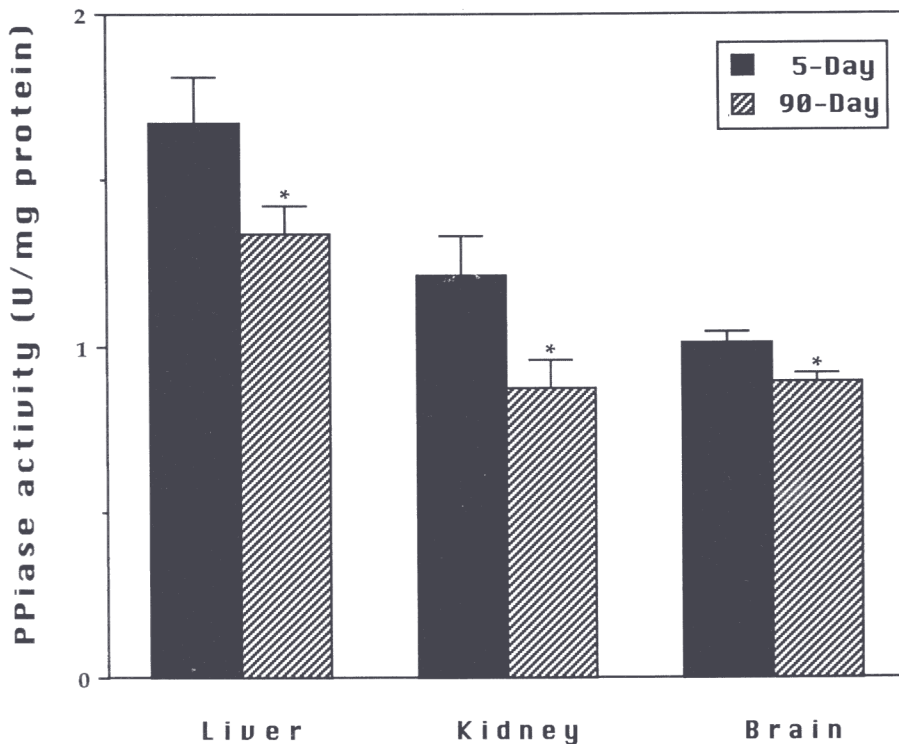


Fig. 1 Activity of PPIase in different tissues of chicken at immature (5-day) and mature (90-day) ages. Values are mean for 4-5 chicken at each age. Bars represent standard deviation. Asterisks indicate statistically significant ( $p < 0.05$ ) values as compared to 5-day.

The role of insulin in chicken growth and development has been earlier suggested (19,20). Our findings indicate that insulin regulation of PPIase activity is age- and tissue- specific. In general, it affects PPIase more at immature age as compared to mature ones. This might be because of changes in the insulin receptor and/or post receptor events in different tissues of chicken at early as well as late postnatal ages (19). Structural differences in the subunit of insulin receptor between liver and brain have also been reported in chicken (20,21). Characterization of specific insulin receptors from kidney suggests a role for insulin on chicken renal function (22). Our findings of inhibitory regulation by hydrocortisone and stimulatory by insulin on the PPIase activity in the liver of immature chicken also corroborate with their antagonistic affects to each other. These findings indicate an age- and tissue- specific regulation of PPIase in the

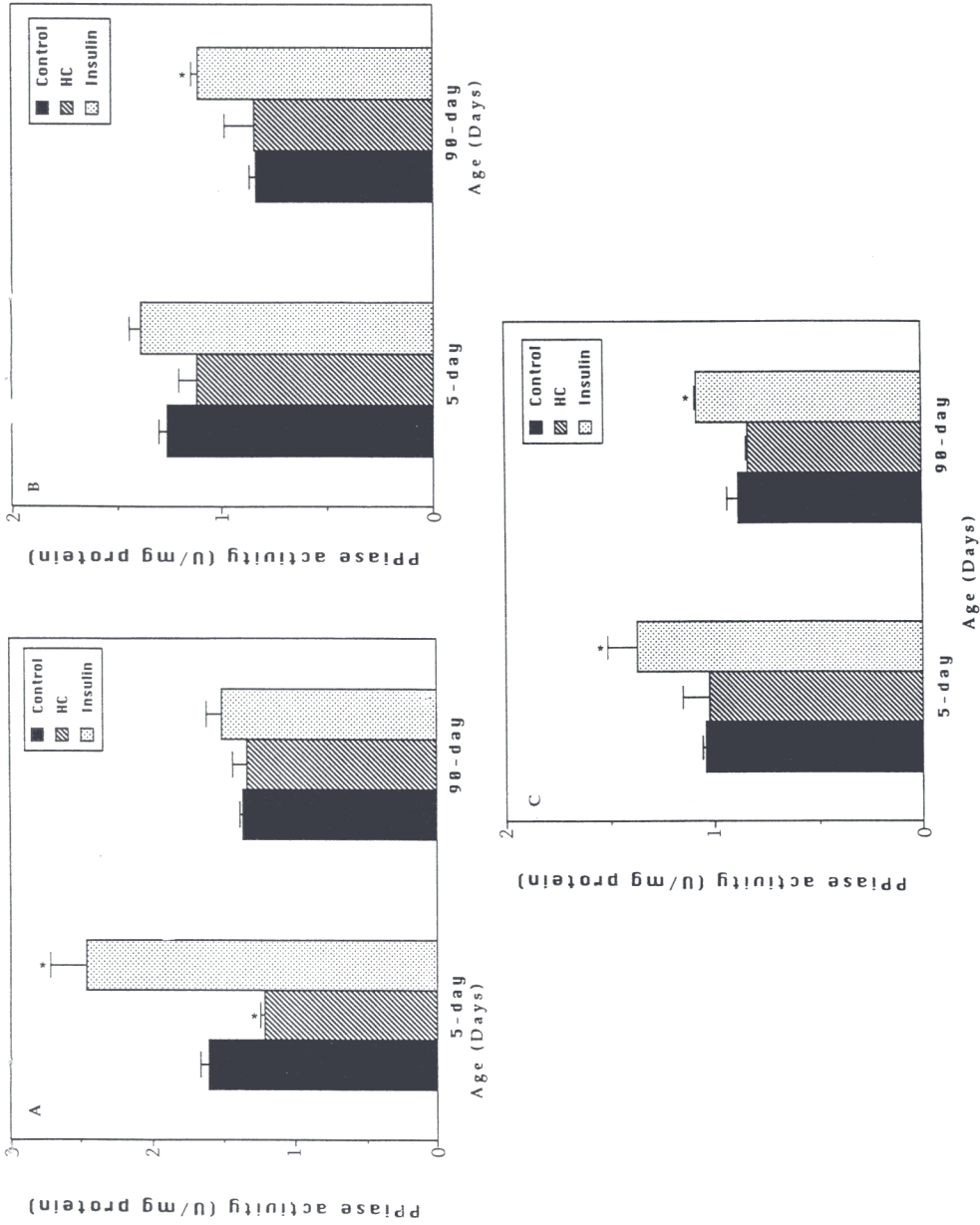


Fig. 2 Effects of hydrocortisone and insulin on the activity of PPIase in the (a) liver, (b) kidney and (c) brain of 5- and 90-day-old chicken. Hormonal treatments and other experimental conditions are described in Materials and Methods section. Values are mean for 4-5 chicken of each age group. Bars exhibit standard deviation. Asterisks indicate statistically significant ( $p < 0.05$ ) values as compared to control.

Table I: Blood sugar levels in immature and mature chicken following insulin administration.				
Parameter	5-day (mg/dl)	% diff.	90-day (mg/dl)	% diff.
Normal	220.6±0.05		258.4±0.01	
		38		30
Test (insulin)	137.3±0.04		182.9±0.03	

Values are mean±SD from 4-5 chicken of each age group.

chicken. Hormonal regulation of chicken liver PPIase at early phases of lifespan may play an adaptive role for such a constitutively expressed enzyme to meet the biosynthetic potential of this tissue.

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