Hydrolysis and Absorption in the Small Intestines of Posthatch Chicks

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ABSTRACT In the immediate posthatch period, chicks must transfer from metabolic dependence on yolk to utilization of exogenous feed. This study describes changes in intestinal luminal pancreatic enzyme activity and mucosal uptake posthatch as influenced by feed and Na intake.

Chicks with access to feed increased in BW and small intestinal weight in the 48-h posthatch, whereas chicks without access to feed decreased in BW; however, small intestinal weight increased during this period.

Chicks ingesting feed showed increases in total intestinal trypsin, amylase and lipase activities that were correlated with intestinal weights and BW. Chicks without access to feed showed little change in trypsin and amylase activities, and these increased only after feed consumption. Feeding a low-Na diet did not significantly change the regression coefficient between pancreatic enzyme activity and BW.

Mucosal uptake was estimated by measuring Na,K+-adenosine triphosphatase (ATPase) activity in small intestinal segments. In fed birds this activity increased in relation to growth, whereas in nonfed birds uptake increased only after access to feed. Low-Na diets allowed only minimal mucosal uptake in all intestinal segments.

This study indicates that secretion of trypsin and amylase into the intestine was triggered by feed intake. In addition, Na plays a critical role in intestinal uptake in the immediate posthatch period.

(Key words: chick, small intestines, posthatch, absorption)

INTRODUCTION

In the initial posthatch period, the young bird must make the transition from metabolic dependence on endogenous lipid-rich yolk to exogenous carbohydrate- and protein-rich feed. This transition is a prerequisite for rapid growth and involves dramatic changes in the gastrointestinal tract, including secretion of digestive enzymes and the initiation of uptake of amino acids and hexoses (Uni et al., 1995).

Hydrolysis of macromolecules in the small intestine is achieved to a large extent by pancreatic enzymes. Net enzyme secretion into the duodenum determined by steady state measurements with nonabsorbed markers from 4-d posthatch indicated that secretion occurs in increasing amounts with age, either due to increased feed intake or to organ size (Noy and Sklan, 1995, Uni et al., 1996). Such measurements cannot be made close to hatch because it is not possible to achieve a steady state, and thus changes in pancreatic enzyme secretions close to hatch have yet to be determined.

Crane (1965) showed that sugar transport into mammalian intestinal epithelial cells is driven by Na-glucose co-transport. More recently, cDNA-encoding, Na+-coupled glucose cotransporters located in the brush border have been cloned (Wright, 1993). In addition, several amino acid transporters with overlapping specificity in the brush border membrane are Na cotransporters (Nakanashi et al., 1994; Munck and Munck, 1999). Once within the cell, homeostasis is maintained by active exclusion of Na+ across the basolateral cell membrane by the Na+,K+ adenosine triphosphatase (ATPase) (Del Castillo and Robinson, 1985), whereas glucose and amino acids are transported out of the enterocyte by passive mechanisms. This transport can be determined by measuring the Na+,K+ ATPase activity in the mucosa. Park et al. (1998) indicated that 31 to 37% of total jejunal O$_2$ uptake was used by the Na+,K+ ATPase. Thus determining either intestinal O$_2$ uptake or Na+,K+ ATPase mucosal activity provides an estimate of Na-dependent intestinal transport.

This paper describes changes in intestinal activity of some pancreatic enzymes and of Na+,K+ ATPase in the small intestines of chicks close to hatch as influenced by feed and Na intake.

MATERIALS AND METHODS

Animals and Treatments

Male Ross × Ross$^2$ broiler chicks were taken immediately following hatch, which was defined as the time

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TABLE 1. Composition of the experimental diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Control (%)</th>
<th>Low-Na (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehulled soybean meal</td>
<td>38.1</td>
<td>38.1</td>
</tr>
<tr>
<td>Corn</td>
<td>32.5</td>
<td>32.9</td>
</tr>
<tr>
<td>Wheat</td>
<td>19.3</td>
<td>19.3</td>
</tr>
<tr>
<td>Oil</td>
<td>6.8</td>
<td>6.8</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.37</td>
<td>. . .</td>
</tr>
<tr>
<td>Vitamin and mineral mix¹</td>
<td>0.23</td>
<td>0.23</td>
</tr>
</tbody>
</table>

¹Provided vitamins and minerals as previously described (Uni et al., 1995).

Chicks with access to feed increased in BW after hatch, whereas chicks without access to feed decreased in BW by 6 g in the 48 h after hatch, but once feed consumption began, they grew at a rate parallel with fed birds (Figure 1). Chicks fed the low-Na diet increased in BW posthatch; however, after 48 h the rate of growth decreased. The rate of yolk utilization was more rapid in chicks with access to feed than either nonfed or low-Na-fed chicks. The weight of the small intestine increased by two-fold in fed chicks and by about 60% in nonfed chicks in the 2 d posthatch. After feed ingestion, the rate of increase in intestinal weight was parallel to fed chicks. Chicks fed the low-Na diet showed lower increases in small intestinal weight from Day 3. Weights of the individual segments of the small intestine (not shown) and total small intestinal weight were closely correlated with BW (BW = 36.2 ± 1.8 + (6.9 ± 0.36)*small intestinal weight, r = 0.94).

Total trypsin, lipase, and amylase activities were determined in the duodenum, jejunum, and ileum. However, because the treatment effects in each individual segment were similar, only total small intestinal activities are

RESULTS

Analyses

Lipase (Belfrage and Vaughan, 1969), trypsin (Sklan and Halevy, 1985), and amylase (Pinchasov and Noy, 1994) activities were determined in the washings from each intestinal segment. The Na⁺,K⁺ATPase activity was assayed as described by Del Castillo et al. (1991) in an incubation medium containing 50 mM Tris (pH 7.2), 5 mM MgCl₂, and 20 mM KCl or 20 mM KCl and 100 mM NaCl, as required, in the presence and absence of 5 mM ouabain. One unit of enzyme activity was defined as the amount of enzyme hydrolyzing 1 mmol of substrate/min under the specified conditions. Sodium was determined by flame photometry.

Statistical Analysis

Least squares means of results are presented after analysis of variance using the general linear models procedures of SAS. Differences between means were tested using t tests, and significance was P < 0.05 unless otherwise stated (SAS Institute, Inc., 1986).
FIGURE 2. Small intestinal trypsin (A), amylase (B), and lipase (C) activity with age in chicks with immediate access to feed (open triangles), held for 48 h without access to feed (circles) or fed a low-Na diet (filled triangles). Results are means ± SD of at least six birds per data point. Non-fed chicks had significantly lower trypsin activity than controls on Days 1 through 4, and low-Na-fed chicks had lower activity on Days 4 and 7. Non-fed chicks had significantly lower amylase activity than controls on Days 1 through 3, and low-Na-fed chicks had lower activity on Days 4 and 7. Non-fed chicks had significantly lower lipase activity than controls from Day 1 through Day 7, and low-Na chicks had lower activity on Days 3 through 7.

In nonfed chicks, small intestinal trypsin and amylase activities were lower prior to feed intake than in fed chicks. Once feeding commenced, activities increased and by Day 7 activities were not different from fed chicks. In contrast, low-Na-fed chicks had similar trypsin and amylase activity to fed birds until 4 d posthatch, after which activity increased more slowly. Lipase activity was similar in all birds until Day 2, after which higher activity was found in fed than nonfed birds. In chicks fed the low-Na diet, lipase activity was lower from Day 3 and remained lower until 7 d.

Total activity of Na⁺,K⁺ ATPase and the ouabain-sensitive Na⁺,K⁺ ATPase activity were determined in the duodenum, jejunum, and ileum. The Na⁺,K⁺ ATPase activity comprised 60 to 75% of total ATPase activity. The activity patterns and effects of the treatments were parallel in the different intestinal segments, and thus only total small intestinal activities are shown (Figure 3). Total small intestinal Na⁺,K⁺ ATPase activity was highest in fed chicks from Days 1 through 4. Nonfed chicks showed low activity before feed ingestion, and then an increase in activity parallel to fed chicks. In contrast, chicks fed the low-Na diet had low activity that increased only slightly with age.

Table 2 indicates the relationships between intestinal enzymatic activities and BW. The pancreatic enzyme activities and the Na⁺,K⁺ ATPase activities were significantly correlated with both BW and intestinal weight (not shown) over 7 d. The regression coefficients were significantly lower for Na⁺,K⁺ ATPase activities in chicks fed the low-Na diet.

DISCUSSION

As in previous studies, holding birds without food for 48 h decreased initial growth until after feed ingestion. However, during the 48 h posthatch, the small intestines increased in weight in all birds; this increase was approximately two-fold in fed birds and about 60% in nonfed birds. Yolk was utilized exponentially during this period at a slightly greater rate in fed than non-fed chicks. These results are similar to those of our previous studies (Noy et al., 1996; Noy and Sklan, 1999) and indicate that in non-fed birds, protein for synthetic and metabolic activities originates from yolk. Energy for maintenance is supplied by the yolk prior to use of exogenous nutrients, which
DIGESTION AND ABSORPTION IN POSTHATCH CHICKS

TABLE 2. Regressions between total intestinal enzymatic activities and BW in posthatch chicks

<table>
<thead>
<tr>
<th>Dependent</th>
<th>Independent</th>
<th>Intercept&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Slope&lt;sup&gt;1&lt;/sup&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipase</td>
<td>BW</td>
<td>−917 ± 315</td>
<td>21.2 ± 4.2</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>Fed</td>
<td>−759 ± 180</td>
<td>18.4 ± 3.2</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>Non-fed</td>
<td>−808 ± 278</td>
<td>19.0 ± 4.1</td>
<td>0.49</td>
</tr>
<tr>
<td>Trypsin</td>
<td>BW</td>
<td>−7,255 ± 1,056</td>
<td>198 ± 14</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>Fed</td>
<td>−10,002 ± 1,152</td>
<td>226 ± 21</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>Non-fed</td>
<td>−10,076 ± 1,719</td>
<td>221 ± 31</td>
<td>0.77</td>
</tr>
<tr>
<td>Amylase</td>
<td>BW</td>
<td>−3,788 ± 1,704</td>
<td>172 ± 23</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>Fed</td>
<td>−4,985 ± 1,709</td>
<td>179 ± 32</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>Non-fed</td>
<td>−5,766 ± 2,154</td>
<td>216 ± 57</td>
<td>0.58</td>
</tr>
<tr>
<td>Na-ATPase</td>
<td>BW</td>
<td>−2.79 ± 1.78</td>
<td>0.29 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>Fed</td>
<td>−4.54 ± 3.30</td>
<td>0.29 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Non-fed</td>
<td>−4.15 ± 3.23</td>
<td>0.19 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.48</td>
</tr>
</tbody>
</table>

<sup>1</sup>Regressions were calculated from data from chicks sampled at 0, 1, 2, 3, 4, and 7 d posthatch; intercepts and slopes are given together with SD.

<sup>a,b</sup>Values without a common superscript differ (P < 0.05).

are available after hydrolysis of feed macromolecules by pancreatic enzymes in the intestine. Activities of the three pancreatic enzymes examined in this study increased with age, although in nonfed chicks trypsin and amylase activities changed little before ingestion of feed. Similar effects of delayed placement on the pancreatic amylase and trypsin activities have been reported in poulets by Corless and Sell (1999). Lipase activity in the intestine is required even before feed ingestion for hydrolysis of yolk triglycerides, and the changes following feed intake observed in the activity of this enzyme in this and previous studies (Uni et al., 1995, 1996) were less dramatic than those observed for trypsin and amylase.

Intestinal pancreatic enzymatic activities were correlated with BW and intestinal weight. In the absence of feed intake and, thus, limited availability of amino acids, synthesis of intestinal tissue has priority over secretion of digestive enzymes. In chicks fed the low-Na diet, BW increase was slower after 48 h, probably due to a decrease in feed intake accompanied by lower secretion of pancreatic enzymes.

In this study, we determined total intestinal activity of pancreatic enzymes by quantitatively removing all of the intestinal contents. Previous determinations of secretion of pancreatic enzymes used steady-state nonabsorbed marker methodology from 4 d posthatch and indicated increases in secretion with age (Noy and Sklan, 1995), but not per gram feed intake (Uni et al., 1995). Marchaim and Kulka (1967) indicated that pancreatic enzymes are present in the small intestines in the late embryonic stages; however, the findings of this study suggest that feed intake triggers enhanced secretion of trypsin and amylase, which are then secreted at relatively constant amounts per feed intake as the chick grows. Because glucose and a large proportion of amino acids are absorbed by co-transport with Na, which is then removed from the enterocyte by active transport, we examined the Na<sup>+</sup>K<sup>+</sup>ATPase activity in different intestinal segments. This mucosal activity was correlated with growth in all intestinal segments, although the slopes differed with intestinal site. Such a relationship is expected between intestinal uptake of major nutrients and growth. In nonfed birds, activity of Na<sup>+</sup>K<sup>+</sup>ATPase was low before feed ingestion, then increased parallel to fed chicks. In contrast, in chicks fed the low Na diet, Na<sup>+</sup>K<sup>+</sup>ATPase activity was strongly depressed, probably due to lack of the Na required for the Na<sup>+</sup>K<sup>+</sup>ATPase activity. This result confirms that a sufficient Na supply is essential for nutrient uptake. Thus it appears that intestinal Na<sup>+</sup>K<sup>+</sup>ATPase activity is a useful parameter for measuring intestinal absorptive activity.

The low in situ uptake of glucose and methionine from yolk previously found close to hatch (Noy and Sklan, 1999) was probably due both to the hydrophobic nature of the substrate and to the low concentration of Na.

Recently, additional Na-dependent transporters have been reported in small intestine brush border membranes, including: L-ascorbic acid (Tsukaguchi et al., 1999), phosphate (Field et al., 1999), multivitamin (Prasad et al., 1999), and taurocholate (Coleto et al., 1998) transporters. Thus Na appears to have a central role in nutrient uptake in the immediate posthatch period and may limit nutrient uptake when Na intake is low.

REFERENCES


