The role of $11\beta$-hydroxysteroid dehydrogenase in maturation of the intestine

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Abstract

Glucocorticoids promote the development of many organs including intestine. At the cellular level, the activity of glucocorticoids is regulated by $11\beta$-hydroxysteroid dehydrogenase ($11\beta$HSD) which converts active glucocorticoids to inactive metabolites. As $11\beta$HSD is also expressed in the intestine, this enzyme may be an important regulator of intestinal maturation. To investigate this, we have performed the systematic study of the development of intestinal $11\beta$HSD activity and its cofactor preference as well as of the effect of $11\beta$HSD inhibition by carbenoxolone on postnatal development of sucrase, alkaline phosphatase and Na,K-ATPase in the intestine. The activity of $11\beta$HSD was low in ileum of suckling rats and significantly increased during the weaning period. In colon, the activity was already high in suckling rats and gradually rose during the postnatal development. $11\beta$HSD activity was undetectable in jejunum both in young and adult rats. At 14.5 nM corticosterone, colonic $11\beta$HSD utilized predominantly NAD as a cofactor, but displayed significant sensitivity also to NADP. Ileal $11\beta$HSD had similar sensitivity to both cofactors. With NAD as a cofactor, ileal $11\beta$HSD had a $K_m$ (59 ± 10 nM) compatible with the colonic enzyme (81 ± 14 nM). Carbenoxolone administration to suckling and weanling rats in vivo did not result in any changes of sucrase activity in jejunum and ileum, alkaline phosphatase activity in ileum and distal colon or Na,K-ATPase activity in ileum. However, carbenoxolone significantly increased Na,K-ATPase activity in distal colon. Our results indicate that the high-affinity type of $11\beta$HSD is expressed not only in colon but also in ileum and that $11\beta$HSD is an important factor in the regulation of tissue levels of active glucocorticoids in developing colon but not in the small intestine. © 1997 Elsevier Science Ireland Ltd.

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1. Introduction

Postnatal maturation of the rodent intestine is characterized by significant modifications of enzyme activities and transport properties. These developmental changes in enzyme status and intestinal transport coincide with weaning period and are influenced by the hormonal milieu of the pups [1,2]. Several lines of evidence indicate that pituitary-adrenal system modulates intestinal development. Adrenalectomy or hypophysectomy of rats attenuate developmental changes and such alterations can be prevented by the administration of corticosterone [1]. Corticosterone, the plasma concentration of which increases just before the time of weaning [3] has been shown to affect the development of various enzymes including sucrase, alkaline phosphatase and peptidases in the small intestine [1,4]. Aldosterone, which has developmental profile similar to corticosterone, induces electrogenic amiloride-sensitive Na⁺ transport in suckling and weanling rats [5].

11β-hydroxysteroid dehydrogenase (11βHSD), a microsomal enzyme responsible for the interconversion of glucocorticoids (corticosterone in the rat), to their inactive 11-dehydro metabolites (11-dehydrocorticosterone in the rat), is considered to be a potential regulator of glucocorticoid and mineralocorticoid action [6,7]. In glucocorticoid-target organs, it might modulate the access of endogenous glucocorticoids to glucocorticoid receptors via 11-dehydrogenase or 11-oxo-reductase pathway. In mineralocorticoid-target tissue, it protects the non-selective mineralocorticoid receptors from glucocorticoid excess. Two species of 11βHSD exist: one is low-affinity and bidirectional (11βHSD1), the other is high-affinity and unidirectional (11βHSD2). 11βHSD1 has higher Kₘ (μM) for its glucocorticoid substrates, preference for NADP/NADPH as cofactors and seems to be an enzyme that produces active glucocorticoids from inactive metabolites in many tissues [8,9]. On the other hand, 11βHSD2 is an enzyme, that has only 11β-dehydrogenase activity, has nanomolar affinity for glucocorticoids and prefers NAD as cofactor [10]. The presence of both isoforms has been demonstrated in multiple organs including rat intestine [11,12]. The intestinal 11βHSD activity is not distributed homogeneously along the intestine and its developmental profile is not identical in particular intestinal segments [13]. After birth 11βHSD activity is high in caecum, proximal and distal colon and does not alter much until adulthood. Quite contrasting developmental profile is typical for ileum, where 11βHSD activity is low during the first two postnatal weeks and increases during weaning period.

The aim of this study was to study cofactor dependence of 11βHSD in the intestinal segments that express different developmental profiles of activity and to
investigate whether 11βHSD plays a physiological role in the maturation of the intestinal epithelium. Using the 11βHSD inhibitor, carbenoxolone [7], we examined the possible role of 11βHSD in the developmental changes of sucrase, alkaline phosphatase and Na,K-ATPase activities during weaning. These enzymes were chosen because they are sensitive to corticosteroids and have distinct developmental profiles. Sucrase and Na,K-ATPase activities increase during development in the small and large intestine (1,14,15) whereas alkaline phosphatase decreases in ileum [16].

2. Materials and methods

2.1. Animals

Wistar rats were bred and maintained on a 12-h light: 12 h darkness cycle and received tap water ad libitum. The day of birth was designated day 0 and approximately 24 h after birth the litter size was reduced to 8 pups which were kept with the dams until they are 30 days old. To examine the effect of carbenoxolone on sucrase, alkaline phosphatase and Na,K-ATPase activities, each litter was divided into two groups. One group received i.p. injections of carbenoxolone (60 mg/kg body wt. day in 0.9% saline) or vehicle only (0.9% saline) from day 12 to day 23. Rats were killed by decapitation and segments of the intestine (jejunum, ileum, colon) were removed, rinsed with ice-cold saline, opened longitudinally and used for enzyme activity studies.

2.2. Assay of 11βHSD activity

The intestinal segments were homogenized in 0.2 M sucrose (1:4 w/v) using a Polytron homogenizer. The homogenate was centrifuged at 1000 × g for 10 min and the supernatant was assayed for protein concentration using the method of Bradford [17]. 11βHSD activity was determined by measuring the rate of conversion of corticosterone to 11-dehydrocorticosterone in assay tubes containing 250 µl of intestinal homogenate (1 mg of protein), 750 µl of buffer (100 mM KCl, 50 mM TRIS-HCl, pH 9.0) and 40 µl NAD or NADP (final concentration 400 µM). After 10 min preincubation at 37°C 1.3 µCi of [3H]corticosterone (final concentration 14.5 nM) was added and the reaction continued 45 min. Preliminary studies indicated that using this amount of tissue protein the rate of reaction was linear within this time. The reaction was terminated by cooling of samples, which were then centrifuged 20 min (3000 × g). The supernatant was loaded onto C18 reverse phase Sep-Pak columns (Waters, Milford, MA, USA) and steroids were quantitatively (98%) eluted in 2 ml methanol. The samples were evaporated to dryness under nitrogen at 40°C, reconstituted in 100 µl methanol, injected onto a steel cartridge (125 × 4 mm internal diameter) packed with LiChrospher 100 RP-18 (5 µm, Merck, Darmstadt, Germany) and analyzed by high performance liquid chromatographic system (Waters, Milford, MA, USA). The samples were separated
using a linear methanol-water gradient from 45:55 (v/v) to 65:35 (v/v) in 15 min and isocratic washing with 100% methanol for 10 min at a flow rate 1.0 ml/min. Column temperature was held at 45°C. The elution of ³H-labelled steroids were monitored by on-line radioactive detection using radioisotope detector with solid cell (Beckman Type 171, Fullerton, CA, USA). After subtraction of background radioactivity, integrated counts within peaks were analyzed by Apex Version 3.1 software (DataApex, Prague, Czech Republic). The activity of 11β-HSD was expressed as the percentage distribution of radioactivity among the HPLC peaks of corticosterone and 11-dehydrocorticosterone in each chromatogram.

Dependence of 11β-HSD activity on pH was measured as mentioned above using pH range of the buffer 7.0–9.5. Reaction was started after 10 min temperature equilibration by adding [³H]corticosterone and continued for another 45 min.

To determine the Kₘ of 11β-HSD for corticosterone, aliquots of intestinal homogenates (ileum 250 μg prot, colon 125 μg prot) were incubated for 10 min (colon) or 20 min (ileum) at 37°C with 14.5 nM [³H]corticosterone and 0.0–1000 nM unlabeled corticosterone and fixed concentration of NAD (400 μM). The data were plotted according to the Lineweaver-Burk double-reciprocal linear transformation of the Michaelis-Menten equation and the least squares best fit computer program was used to calculate the Kₘ and Vₘₐₓ values.

2.3. Assay of sucrase

Sucrase was assayed in mid-jejunum and mid-ileum. For this purpose the segments were slit lengthwise and scraped with a steel spatula to remove mucosa. Homogenate of mucosa was prepared in 9 volumes of 154 mM KCl using a Teflon homogenizer. Sucrase activity was determined in 60 mM sodium maleate buffer (pH 6.0) with 300 mM sucrose at 37°C. The reaction was stopped by boiling and the glucose liberated was measured with the glucose oxidase reagent. Corrections were made for endogenous glucose in the tissue and in the substrate. Sucrase activity was expressed as μmoles of glucose produced per hour and mg of protein. Protein was measured by the method of Bradford [17].

2.4. Assay of Na,K-ATPase

Na,K-ATPase activity was measured in crude homogenate as the ouabain-sensitive release of inorganic phosphate from ATP [14]. Briefly, the mucosa was scraped and homogenized with a Teflon pestle in nine volumes of the solution containing (mM): 30 TRIS-HCl; 250 sucrose; 5 Na₂EDTA; pH 7.3. Samples of homogenate were preincubated at 37°C for 10 min in a solution containing (in mM): 100 NaCl; 100 TRIS-HCl; 20 KCl; 5 MgCl₂; pH 7.3 with or without ouabain (final concentration 2 mM). The reaction was started by addition of ATP (final concentration 3.2 mM) and continued for 30 min. After stopping the reaction by trichloroacetic acid, the released inorganic phosphate was assayed. Na,K-ATPase activity was calculated as the difference between ATPase activity without and with ouabain and expressed as μmol of inorganic phosphate per mg of protein per hour.
2.5. Alkaline phosphatase

Alkaline phosphatase was measured according to Murer et al. [18]. Samples of homogenate were added to a mixture containing (in mM): 50 glycine-NaOH buffer (pH 10.5); 2 MgSO₄ and 2.5 ZnSO₄. After a 10-min preincubation at 37°C the reaction was initiated by addition of p-nitrophenyl phosphate as substrate (final concentration 5.5 mM). The reaction was stopped with 2 M NaOH. The activity was expressed as μmol of released p-nitrophenol per mg of protein per hour.

2.6. Statistical analysis

The data are presented as the means ± S.E.M. Group data were compared using Student’s t-test. Statistical significance was set at P < 0.05.

3. Results

The developmental profile of 11βHSD activity (Fig. 1) and cofactor preference were investigated in homogenates of three intestinal segments - jejunum, ileum and colon. Colon is the site of a high 11βHSD activity which increases only moderately during development. In contrast, ileum of suckling rats has very low activity which is much higher in weaning period and adulthood. The conversion of corticosterone is absent in jejunum. At 14.5 nM corticosterone, the colonic 11βHSD has a clear preference for NAD over NADP activity in both young and adult animals. The enzyme used NADP to dehydrogenate corticosterone at 50% rate compared to that observed when NAD was used as a cofactor. The rate of conversion was very low in the absence of cofactors in all three segments. (Fig. 1). The effect of pH on the activity of 11βHSD is shown in Fig. 2. Unlike the NAD-dependent 11βHSD from cultured human colonic cells T84, which has a pH optimum of approximately seven [19], the rat colonic enzyme had a pH optimum of about nine. Carbenoxolone (0.1 M) strongly inhibited 11βHSD in both young (82 ± 9%) and adult (88 ± 10%) rats. The kinetic analysis of 11βHSD activity in ileum and colon revealed the presence of high-affinity 11βHSD in both intestinal segments (Table 1). Maximal capacity (Vmax) was, however, attenuated in ileum.

To investigate whether 11βHSD could play a role in the developmental changes of enzymes that are known to be sensitive to corticosteroids, we examined Na,K-ATPase and alkaline phosphatase activities in ileum and distal colon and sucrase activity in jejunum and ileum. As carbenoxolone was demonstrated to be efficient to inhibit 11βHSD (see above), the developmental changes of these enzymes were examined in the presence and absence of carbenoxolone in 24-day-old rats. The reason for the study of jejunum, which has no dehydrogenase activity (Fig. 1), was the finding of considerable 11-oxo-reductase activity in adult jejunum [20]. The data in Tables 2 and 3 show that carbenoxolone did not change neither the activity of alkaline phosphatase in colon and ileum nor the activity of sucrase in jejunum and ileum. In contrast to sucrase and alkaline phosphatase, carbenoxolone administra-
Fig. 1. 11β-hydroxysteroid dehydrogenase activity in the presence of 14.5 nM corticosterone and its dependence on cofactors (NAD or NADP). Data are means ± S.E.M. (n = 5). The activity was expressed as conversion of corticosterone to 11-dehydrocorticosterone. Significantly different from the data obtained in the presence of NADP (*P < 0.05).
Fig. 2. Effect of pH on activity of 11β-hydroxysteroid dehydrogenase in distal colon of adult rats expressed as conversion of corticosterone to 11-dehydrocorticosterone in the presence of NAD.

ation resulted in significant changes of distal colonic Na,K-ATPase but not of ileal Na,K-ATPase (Table 3). As shown in Fig. 3, the sucrase and Na,K-ATPase activities rose in intact rats during postnatal development whereas alkaline phosphatase activity declined.

4. Discussion

In the previous study we have demonstrated the different developmental profile of 11β/HSD in ileum and colon and shown that corticosteroids stimulate 11β/HSD activity in immature and adult intestine [13]. This study is the first attempt to evaluate the possible role of 11β/HSD action in maturation of the intestine. Since

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$K_m$ (nM)</th>
<th>$V_{max}$ (pmol/min x mg protein)</th>
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</thead>
<tbody>
<tr>
<td>Ileum</td>
<td>59 ± 10</td>
<td>66 ± 6</td>
</tr>
<tr>
<td>Distal colon</td>
<td>81 ± 14</td>
<td>8 ± 1</td>
</tr>
</tbody>
</table>

Kinetic study was performed in the presence of NAD as described in Section 2, 24-day-old rats were used. Values are means ± S.E.M. of four separate experiments.
Fig. 3. Developmental changes of sucrase, Na,K-ATPase and alkaline phosphatase activities in jejunum, ileum and distal colon of intact rats during weaning. Each point is the mean ± S.E.M. (sucrase: n = 10–17; Na,K-ATPase: n = 6–8; alkaline phosphatase: n = 8–10).
The data are mean ± S.E.M., sucrase activity in μmol glucose/(h × mg protein), numbers of animals in parentheses. Controls, 24-day-old rats that were i.p. treated with vehicle since day 12; carbenoxolone treated rats received the drug in a dose 60 mg/(kg body wt. day) since day 12 of life.

Table 2
Effect of in vivo carbenoxolone administration on the activity of sucrase in jejunum and ileum

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Carbenoxolone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum 5.34 ± 0.79 [14]</td>
<td>6.71 ± 0.65 [15]</td>
<td></td>
</tr>
<tr>
<td>Ileum  1.61 ± 0.36 [15]</td>
<td>1.81 ± 0.28 [22]</td>
<td></td>
</tr>
</tbody>
</table>

glucocorticoids are essential for maturation of intestine [1] and 11βHSD regulates the availability of glucocorticoids in target tissues and cells [21–23], 11βHSD might play an important role in the intestinal development. In addition, 11βHSD has been proposed to protect mineralocorticoid receptors from binding glucocorticoids in mineralocorticoid-target tissue including distal colon [28] and immature distal colon has significantly increased Na⁺ absorption due to stimulation of electrogenic Na⁺ transport pathways by aldosterone [5]. However, the precise physiological role of 11βHSD during development remains speculative.

We have demonstrated that the developmental profile of 11βHSD activity in ileum is different from the profile in caecum and proximal or distal colon. The ileal developmental profile was comparable with that of 11βHSD1 isoform in liver [24], whereas the profile in the large intestinal segments was very similar to the profile in kidney [25] which is the major site of 11βHSD2 biosynthesis in adult animals [26]. The present results demonstrate clear-cut difference in cofactor preference in ileum and distal colon and indicate the existence of both isoforms in the intestine. Kₘ for rat liver 11βHSD1 is approximately 1 μM [9] and NAD has no significant effect on its activity [27]. In contrast, 11βHSD2 in colonic T84 cells has Kₘ 11 nM and clear preferences for NAD over NADP [19]. As we used substrate concentration 14.5 nM, our data seem to reflect the presence of 11βHSD2 in both ileum and distal colon. This conclusion is supported by the kinetic analysis (Table 1) which has demonstrated the presence of a high-affinity ileal and colonic 11βHSD2 similar to that recently reported in adult rat and human surface colonocytes [12]. The ‘mixed’

Table 3
Effect of in vivo carbenoxolone administration on the activities of alkaline phosphatase and Na,K-ATPase in ileum and distal colon of young rats

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Treatment</th>
<th>Ileum</th>
<th>Distal colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline phosphatase</td>
<td>Controls</td>
<td>17.1 ± 3.0 [9]</td>
<td>3.6 ± 0.6 [11]</td>
</tr>
<tr>
<td></td>
<td>Carbenoxolone</td>
<td>17.7 ± 4.6 [11]</td>
<td>3.8 ± 0.5 [15]</td>
</tr>
<tr>
<td>Na,K-ATPase</td>
<td>Controls</td>
<td>6.55 ± 0.69 [14]</td>
<td>4.03 ± 0.59 [13]</td>
</tr>
<tr>
<td></td>
<td>Carbenoxolone</td>
<td>6.22 ± 1.05 [15]</td>
<td>5.71 ± 0.42* [16]</td>
</tr>
</tbody>
</table>

Alkaline phosphatase in μmol of p-nitrophenol produced per hour and mg protein; Na,K-ATPase activity in μmol of phosphate produced per hour and mg protein. For further details see Table 2.

*Significantly different from control animals (P < 0.05).
cofactor preference probably reflects the presence of both isoforms of 11\(\beta\)HSD in the intestine as was recently demonstrated in distal colon of adult rats [12]. According to these findings 11\(\beta\)HSD2 is localized predominantly in the surface colonocytes which are responsible for \(\text{Na}^+\) transport and are target cells for mineralocorticoid action, whereas 11\(\beta\)HSD1 is expressed predominantly in subepithelial cells. The question which is not yet resolved is the reversibility of colonic 11\(\beta\)HSD1 isoform. Whorwood et al. [12] observed in isolated colonic subepithelial cells 11-oxo-reductase activity in the presence of 500 nM 11-dehydrocorticosterone but we have not seen any 11-oxo-reductase activity in slices of ileum and colon in the presence of 1.45 \(\mu\)M 11-dehydrocorticosterone [13,14]. In contrast, Marhefka et al. [20] detected 11-oxo-reductase activity in slices of duodenum, jejunum and ileum of adult rats. At present it is no explanation for these differences.

The results discussed above support the view that intestinal isoforms of 11\(\beta\)HSD possess dehydrogenase activity and perhaps also oxo-reductase activity, i.e. they can increase or decrease glucocorticoid exposure to the glucocorticoid and mineralocorticoid receptors. However, the physiological significance of these processes remains to be fully defined. Postnatal maturation of the rodent intestine is characterized by significant modifications of enzyme status during the weaning period when it is modulated by corticosteroids [1,2]. To test the functional role of 11\(\beta\)HSD in developing intestine, we have investigated the effect of 11\(\beta\)HSD inhibitor carbenoxolone on the activity of three enzymes with large developmental changes during the weaning period which are sensitive to corticosteroids. These enzymes were sucrase, the activity of which substantially increases during weaning in jejunum and ileum [1]; alkaline phosphatase, whose activity decreases in ileum during weaning [16] and \(\text{Na,K-ATPase}\) whose activity rises postnatally in colon [14] and in ileum [15]. Even if sucrase and alkaline phosphatase activities are increased by glucocorticoids [1,29], we have not observed any significant changes of their activity in carbenoxolone-treated animals. In contrast, carbenoxolone administration significantly increased colonic but not ileal \(\text{Na,K-ATPase}\). This is in agreement with the findings that, in immature rats, colonic \(\text{Na,K-ATPase}\) is increased by mineralocorticoids [31] and glucocorticoids [30] but not with the data of Zemelman et al. [15] who demonstrated the stimulatory effect of glucocorticoids on \(\text{Na,K-ATPase}\) in immature ileum. Similar effect of carbenoxolone on \(\text{Na,K-ATPase}\) was also observed in adult kidney [28,32]. The absence of the effect of carbenoxolone in ileum indicates that 11\(\beta\)HSD has a protective effect on \(\text{Na,K-ATPase}\) only in mineralocorticoid-sensitive tissue. The activity of 11\(\beta\)HSD in colonocytes of the immature rats may protect these cells from glucocorticoids and support the mineralocorticoid regulation of \(\text{Na}^+\) transport via \(\text{Na}^+\) channels and \(\text{Na,K-ATPase}\) [5,31]. The developmental increase of colonic \(\text{Na,K-ATPase}\) seems to be mineralocorticoid-and 11\(\beta\)HSD-dependent whereas the glucocorticoid-dependent development of intestine does not seem to be influenced by this enzyme.
Acknowledgements

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References


