



DISTRIBUTION OF 11β -HYDROXYSTEROID DEHYDROGENASE ALONG THE RAT INTESTINE

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Summary

Aldosterone selectivity of mineralocorticoid target tissues has been suggested to be due to the inactivation of glucocorticoids in the target tissue by 11β -hydroxysteroid dehydrogenase (11β -OHSD). The distribution of 11β -OHSD was studied in the intestine which is composed of aldosterone-sensitive and insensitive segments. The activity of the enzyme was high in distal colon and medium in ileum, cecum, and proximal colon. Zero activity was found in duodenum and jejunum. Carbenoxolone completely blocked the enzyme. Low-salt diet increased the activity in proximal colon and decreased in ileum. Adrenalectomy decreased the activity in ileum and proximal colon. The existence of segmental differences in the distribution of 11β -OHSD and the hormonal effect on the activity of the enzyme suggest a physiological role of 11β -OHSD in the intestine.

The enzyme 11 -hydroxysteroid dehydrogenase (11β -OHSD, E.C.1.1.1.146) has been suggested to be the key factor responsible for the tissue sensitivity to aldosterone (1,2). This enzyme converts glucocorticoids (corticosterone, cortisol) to their 11 -keto analogs (11 -dehydrocorticosterone, cortison) which seem to be biologically inactive derivatives. Since, *in vitro*, aldosterone and glucocorticoids bind to the intestinal and renal mineralocorticoid receptors with the similar affinity (2,3) and the plasma level of glucocorticoids is much higher than that of aldosterone, the activity of 11β -OHSD might prevent the occupancy of the mineralocorticoid receptors by glucocorticoids. To conclude that 11β -OHSD is responsible for the protection of mineralocorticoid receptors, it is necessary to establish that this enzyme is localized in aldosterone target tissues. Intestinal epithelia are either aldosterone-sensitive (colon), or -insensitive, e.g. jejunum (4,5,6). However, all of these segments were demonstrated to possess mineralocorticoid receptors (7). The goal of our study was to test whether the distribution of 11β -OHSD follows the tissue sensitivity to aldosterone or the distribution of mineralocorticoid receptors and whether changes of corticosteroid status may influence the activity of the enzyme.

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Material and Methods

Experiments were performed on male Wistar rats, body weight 250 - 300 g, that were fed a standard diet. The animals were divided into 3 groups, the control, Na⁺-deficient, and adrenalectomized rats. The control group was maintained on normal chow containing 17 mEq Na⁺ 100 g⁻¹ and the Na⁺-deficient animals on low-salt diet (ICN cat. no. 902902, \geq 0.8 mEq Na⁺ 100 g⁻¹, 9 days). Animals tolerated both diets well and the food consumption was 5.4 \pm 0.3 g/100g BW.day in control group and 5.6 \pm 0.5 g/100g BW.day in Na⁺-deficient animals. The corresponding Na⁺ intake was 911 μ Eq Na⁺/100 g BW.day and less than 44 μ Eq Na⁺/100 g BW.day, respectively. The third group was bilaterally adrenalectomized 4 days before the experiments. Thereafter, the rats had a free access to 0.9 % NaCl as drinking fluid and normal chow. During anaesthesia blood was withdrawn from the abdominal aorta, then the rats were sacrificed, the segments of the intestine removed, opened longitudinally and the gut content rinsed off in the ice-cold incubation solution containing (in mM) Na⁺, 140.0; K⁺, 5.4; Ca²⁺, 1.2; Mg²⁺, 1.2; Cl⁻, 123.8; HPO₄²⁻, 2.4; H₂PO₄⁻, 0.6; HCO₃⁻, 21.0; glucose, 10.0; β -hydroxybutyrate, 0.5; glutamine, 2.5; and mannitol, 10.0 previously gassed for 10 minutes with 95 % O₂/ 5 % CO₂. Plasma aldosterone was measured by radioimmunoassay (Adico Ltd., Prague, Czech Republic) in 100 μ l triplicates of the plasma.

11 β -OHSD assay: As 11 β -OHSD complex possesses not only 11 β -dehydrogenase but also 11-oxoreductase activity (11-dehydrocorticosterone to corticosterone), both activities were measured. Sheets of tissue were incubated for 2 h at 37°C in oxygenated incubation solution with corticosterone or 11-dehydrocorticosterone SIGMA (50 μ g/100 ml). In the experiments where the inhibition of 11 β -OHSD by carbenoxolone was studied, the tissues were preincubated 30 min in the presence of 0.8 mM carbenoxolone before corticosterone was added and then the tissue was incubated additional 90 minutes. The concentration of carbenoxolone was chosen according to Kenouch et al. (8) because this concentration results in an almost complete inhibition of 11 β -OHSD in renal tubules. At the end of the incubation, the tissue was removed and the solution was shaken vigorously with ethyl acetate that was then removed by evaporation under vacuum. The amounts of 11-dehydrocorticosterone, DCS and corticosterone, CS were determined by HPLC as described later. The results are expressed as the conversion rate of corticosterone to 11-dehydrocorticosterone [DCS/(DCS + CS)] per g of dry weight per 2 hours.

High-performance liquid chromatography: HPLC was carried out with a Waters automated gradient controller (Millipore, Milford, MA, USA) with Waters Model 510 pump. The steel column (250 x 4 mm I.D.) packed with Separon SGX 7 μ m (Tessek, Prague, Czech Republic) and glass precolumn with Separon SGX 5 μ m were mounted in the instrument. The sample was dissolved in 300 μ l methanol and 90 μ l was injected into the column. Elution was under linear methanol-water gradient from 45:55 (v/v) to 65:35 (v/v) in 20 min; isocratic washing with 100% methanol followed for another 10 min. The flow-rate was 1.0 ml/min and the column temperature was held at 45°C. The eluate was monitored at 243 nm using a SpectraPhysics SP 8400 detector (San Jose, CA, USA) and its signal was computer processed by software Apex v2.0 (DataApex, Prague, Czech Republic). Retention time of 11-dehydrocorticosterone was 14.2 min and corticosterone 16.8 min. Calibration was made at range 0.3 - 3.0 μ g per injection with cubic regression (correlation coefficient was 0.999 at both cases). The limit of detection for both corticosteroids was 0.02 μ g.

Statistical analysis: All data were expressed as means \pm SEM and compared by Student's *t* test. A probability value < 0.05 was considered significant.

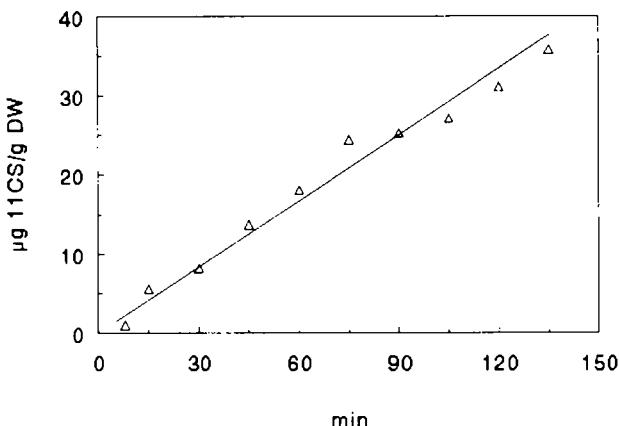


FIG. 1.

Time course of conversion of corticosterone to 11-dehydrocorticosterone (11CS) in distal colon. The sheets of tissue (35 ± 3 mg of dry weight) were incubated at 37°C at an initial corticosterone concentration $5 \mu\text{g}/10 \text{ ml}$. The samples were taken consecutively after 8 and 15 min and analyzed using HPLC.

Results and Discussion

The time course of 11-dehydrocorticosterone formation is shown in Fig. 1. Using the substrate concentration of corticosterone $5 \mu\text{g}/10 \text{ ml}$ the 11β -dehydrogenase activity is linear between 8 and 235 minutes (Fig. 1). Since reduction of 11-dehydrocorticosterone to corticosterone was below the limit of detection after 2 hours of incubation of the tissue in the presence of 11-dehydrocorticosterone ($5 \mu\text{g}/10 \text{ ml}$) we assume that the 11β -dehydrogenase activity is not underestimated due to back-conversion of 11-dehydrocorticosterone to corticosterone.

The distribution of 11β -OHSD in various intestinal segments is given in Table 1. These results indicate different activity of the enzyme along the intestine. The activity is very low or zero in the proximal parts of the intestine, medium in ileum, cecum, and proximal colon and very high in distal colon. The distribution of 11β -OHSD reflects the distribution of aldosterone target epithelia and not of the mineralocorticoid receptors. Mineralocorticoid receptors were demonstrated in duodenum, jejunum, ileum and colon (7), but typical aldosterone-sensitive segments are only colon and cecum (15). In these segments aldosterone increases Na^+ absorption, K^+ secretion and activity of Na,K -ATPase (4,5,9-11). Mineralocorticoid regulation of Na^+ transport was not demonstrated in small intestine, especially in duodenum and jejunum (15). However, in lower part of the small intestine, ileum, aldosterone exerts modest effect on electrolyte transport (6). The regulatory role of aldosterone in this intestinal segment is in accordance with our finding of 11β -OHSD activity in ileum (Table 1).

Carbenoxolone, an inhibitor of 11β -OHSD (12), totally inhibited the conversion of corticosterone to 11-dehydrocorticosterone in ileum, cecum, proximal and distal colon. The present of 11-dehydrocorticosterone was below our limit of detection, i.e. the

TABLE 1

Effect of adrenalectomy and Na deficiency on the activity of 11β -hydroxysteroid dehydrogenase

Segment	Treatment		
	Control	Adx	Na def.
duodenum	0.00	0.00	0.00
jejunum	0.17 ± 0.10 ⁺	0.12 ± 0.04 ⁺	0.02 ± 0.05 ⁺
ileum	2.93 ± 0.31	0.86 ± 0.22 [*]	2.16 ± 0.11 [*]
cecum	4.42 ± 0.68	3.98 ± 0.58	3.23 ± 0.42
proximal colon	3.49 ± 0.09	2.31 ± 0.29 [*]	4.83 ± 0.43 [*]
distal colon	7.30 ± 0.52 ^{**}	6.19 ± 0.31 ^{**}	8.30 ± 0.30 ^{**}

Values are means ± SEM (n=3-5). The activity of the enzyme was determined as described in METHODS and is expressed as the conversion of corticosterone to 11-dehydrocorticosterone [DCS/(DCS + CS)] per g of dry weight per 2 hours. Treatments indicated are as follows: control, normal diet containing Na; Adx, adrenalectomized rats 4 days before the experiments; Na def., low-salt diet and distilled water for 9 days. ⁺Activity not significantly different from zero ($P < 0.05$), significant differences ^{*}from control rats and ^{**}from ileum, cecum and proximal colon ($P < 0.05$).

11β -OHSD activity in the presence of carbenoxolone was less than 0.21 per g of dry weight per 1.5 h (range 0.21-0.06) in all segments. These results are in accordance with those of Funder et al. (2) in the colon of neonatal rats. Since 11β -OHSD is thought to have a local effect, one can expect that its activity might be modified during the changes of corticosteroid status of the organism (13). As shown in Table 1, the activity of 11β -OHSD was significantly increased during secondary hyperaldosteronism only in the proximal colon but not in other segments (plasma level of aldosterone was 154 in control and 2760 pg/ml in Na^+ -deficient groups). In contrast, adrenalectomy decreased the activity in ileum and proximal colon (Table 1). Both maneuvers did not exert any effect in duodenum and jejunum. One explanation of these findings may be that there are multiple forms of the enzyme present in the intestine and that only some of them are steroid-dependent. Recent findings of Krozowski et al. (16) demonstrated that low-salt diet elevates 11β -OHSD mRNA only in some tissues and not in others and the data of Hammami and Siiteri (13) indicate that 11β -OHSD is under multifactorial regulation including glucocorticoids.

The reported distribution of 11β -OHSD along the intestine provides a strong evidence for the view that the rat intestinal 11β -OHSD is regulated by corticosteroids and that this enzyme determines the tissue specificity for aldosterone. The high activity of 11β -OHSD in the distal colon explains why the colonic mineralcorticoid receptors discriminate between aldosterone and corticosterone. Incubation of colonic mucosa with aldosterone but not with corticosterone induced amiloride-sensitive Na^+ transport (12). However, corticosterone induced amiloride-sensitive Na^+ transport in the presence of an inhibitor of 11β -OHSD (12). ^3H -aldosterone binding to colonic mineralcorticoid receptors *in vivo* is more than one order of magnitude higher than that of ^3H -corticosterone (14) but the binding to isolated colonic cytosol is approximately the same (3).

In summary, we have characterized the distribution of 11 β -OHSD in the intestine and shown for the first time the influence of corticosteroid status of the organism on the activity of the enzyme in some intestinal segments.

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