ELSEVIER

PII S0024-3205(97)00956-9

Life Sciences, Vol. 61, No. 24, pp. 2391-2396, 1997

0024-3205/97 $17.00 + .00

HORMONAL REGULATION OF INTESTINAL 11β-HYDROXYSTEROID DEHYDROGENASE

J. Pácha[1], I. Mikšík, V. Lisá and I. Pohlová

Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic

(Received in final form September 23, 1997)

Summary

We have previously demonstrated the developmental increase of the activity of 11β-hydroxysteroid dehydrogenase (11βHSD) in the rat ileum which correlated with the developmental surge of plasma concentrations of corticosteroids, thyroid hormones and insulin. To ascertain whether these hormones directly stimulate 11βHSD activity we used explant cultures of ileum and distal colon. The intestinal segments of young, 7-day-old rats, were cultured 48 hours in the presence of aldosterone (10^{-7} M), dexamethasone (10^{-7} M), triiodothyronine (10^{-7} M) or insulin (10^{-7} M) and 11βHSD activity was evaluated by measuring the conversion of [^{3}H]corticosterone to [^{3}H]11-dehydrocorticosterone. The activity of 11βHSD was significantly increased following 48 h treatment with dexamethasone and aldosterone, whereas insulin and triiodothyronine were without any effect. Corticosterone oxidation was inhibited by carbenoxolone and progesterone. It is being concluded, that both glucocorticoids and mineralocorticoids but not insulin or triiodothyronine induce intestinal 11βHSD activity.

Key Words: 11β-hydroxysteroid dehydrogenase, hormonal regulation, intestine, mineralocorticoid, glucocorticoid

The enzyme 11β-hydroxysteroid dehydrogenase (11βHSD) catalyses the inactivation of the principal glucocorticoids (cortisol, corticosterone) to their inert 11-keto metabolites (cortisone, 11-dehydrocorticosterone). The primary function of this enzyme is assumed to be the protection of mineralocorticoid receptors against the binding of glucocorticoids (1,2). It is now widely accepted that there are at least two isoforms of 11βHSD. The first is a NADP/NADPH dependent isoform (11βHSD1) that was purified from rat liver (3) and whose corresponding cDNA has been cloned

[1]Correspondence: J. Pácha, Institute of Physiology, Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, Czech Republic. Fax: +4202-4719517, E-mail:pacha@biomed.cas.cz

(4). This enzyme contains both dehydrogenase and reductase activities and has K_m in the μM range. The second, a more recently identified isoform, is a NAD-dependent isoform (11βHSD2) which has only 11β-dehydrogenase activity and high affinity for the substrate (K_m in the nM range) (5,6) and was recently cloned (7,8,9). This isoform is present in the placenta and in aldosterone target tissues where it protects mineralocorticoid receptors from glucocorticoid binding (10). Even if 11βHSD expression is widespread, little is known about the factors that regulate this expression. Marked variations in enzyme activity during ontogenesis (11,12) and in response to glucocorticoids, insulin, sex hormones and thyroid hormones in various organs and tissues (13,14,15,16) indicate the possibility of organ-specific regulation of 11βHSD and/or different regulation of both isoforms of 11βHSD.

In the present report, we addressed the question of the regulation of intestinal 11βHSD in the ileum and distal colon which express different developmental patterns of 11βHSD activity. We have previously demonstrated that colonic 11βHSD activity is already high during the neonatal period and does not change appreciably during development. However, in the ileum, the activity of 11βHSD is very low during the first two postnatal weeks then it rises to a peak in 4-week-old rats before falling to a plateau level. The developmental profile of ileal 11βHSD activity (12) correlates with the developmental profiles of plasma levels of corticosterone (17), aldosterone (18), thyroid hormones (19) and insulin (20). In view of these hormonal changes, our work was performed to examine whether the developmental surge of corticosteroids, insulin and thyroid hormones can be accounted for the developmental changes of 11βHSD and whether the hormonal effect is mediated indirectly or directly in the intestine.

Materials and Methods

Seven-day-old rats were decapitated and the distal colon and ileum were removed aseptically and washed in phosphate-buffered saline containing 80 μg/ml gentamycin. The intestinal segments were opened longitudinally and cut with a razor blade into small fragments. The fragments of ileum and distal colon (1.11 ± 0.02 mg dry wt) were placed into an uncoated bacteriological culture dish (diameter 60 mm, KOH-I-NOOR, Czech Republic) and cultured at 37°C under 5 % CO_2 and 95 % O_2 in humidified atmosphere for 48 hours (21). The explants were maintained in 4 ml of Dulbecco's modified Eagle's medium (4.5 g/l glucose, 0.58 g/l L-glutamine; Sevac, Prague, Czech Republic) supplemented with 40 μg/ml gentamycin and 5 % foetal bovine serum. The villus and crypt architecture was well preserved during the time of incubation. The explants were incubated without or with insulin, triiodothyronine or corticosteroids. Hormones were added from concentrated stock solutions to reach the final concentration (in M): insulin, 10^{-7}, triiodothyronine, 10^{-7}, aldosterone 10^{-7}, and dexamethasone, 10^{-7}. The concentrations of insulin, dexamethasone and triiodothyronine were identical to those previously described in studies of hormone action on 11βHSD in fibroblasts, hepatocytes and pituitary GH_3 cells (13, 14, 22). Furthermore, Gaeggeler et al. (23) demonstrated that the maximum stimulatory effect of aldosterone and corticosterone on electrogenic Na^+ transport of urinary bladder cell line is reached at the concentration 10^{-7} M.

11βHSD activity was measured in one-day-old explant cultures by the incubation of the explants with 1.3 x 10^{-8} M [^{3}H]corticosterone added to the culture medium without or with carbenoxolone (5 x 10^{-6} M) or progesterone (5 x 10^{-6} M). After 24 hours of the incubation with [^{3}H]corticosterone, the explants were removed, the samples of the culture media were loaded onto C18 reverse phase Sep-Pak cartridges (Waters, Milford, MA, USA) and the steroids were eluted in 2 ml methanol. The samples were evaporated to dryness under nitrogen at 40 °C, reconstituted in

100 µl methanol and 20 µl was injected onto a Lichrospher 100 RP-18 (125 x 4 mm column, 5 µm; Merck, Darmstadt, Germany). The samples were eluted using a linear methanol-water gradient (Waters, Milford, MA, USA) from 45:55 (v/v) to 65:35 (v/v) in 15 min followed by isocratic washing with 100% methanol for 10 min at a flow rate 1.0 ml/min. The column temperature was held at 46°C and the elution of ^{3}H-labelled steroids was monitored by on-line radioactive detection using a 171 Radioisotope detector with a solid cell (Beckman, Fullerton, CA). After subtraction of background radioactivity, the integrated counts within peaks were analyzed by Apex v3.1 software (DataApex, Prague, Czech Republic). The activity of 11βHSD was expressed as the percentage of distribution of radioactivity among the HPLC peaks in each chromatogram. The results were expressed as means ± SEM. Statistical significance was assessed by Student's *t*-test. $P<0.05$ was considered significant.

Results and Discussion

The activity of 11βHSD decreased significantly during experimental incubation (not shown) but it was clearly evident in all tissues examined. The decrease of activity of ileal 11βHSD after 48 hours of incubation exceeded that of colonic 11βHSD. Nevertheless both explant cultures of distal colon and ileum converted corticosterone to 11-dehydrocorticosterone. In the distal colon, nearly 13% of corticosterone was converted after 24 hours (Fig.1). In the ileum, the conversion of

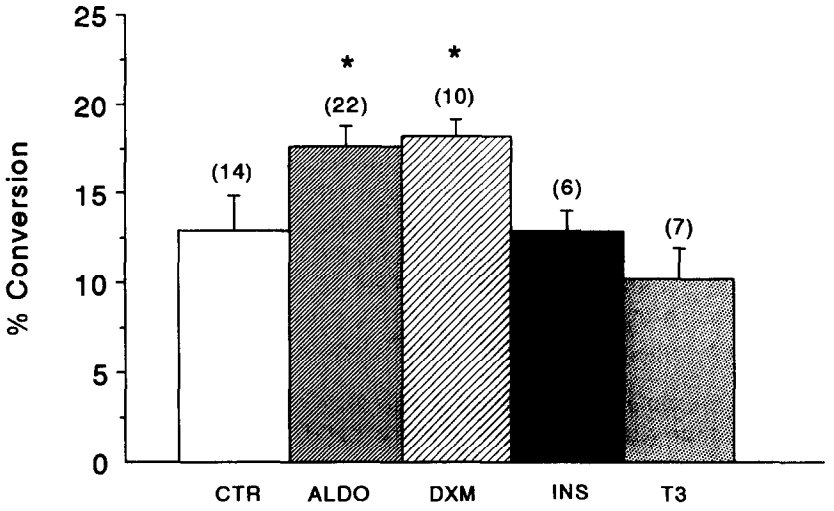

Fig. 1

Effect of corticosteroids, insulin, and triiodothyronine on 11βHSD activity in the distal colon. The explant cultures were incubated in a culture medium containing vehicle (CTR); 10^{-7} M aldosterone (ALDO), 10^{-7} M dexamethasone (DXM), 10^{-7} M insulin (INS) or 10^{-7} M triiodothyronine (T_3). Activity of 11βHSD is expressed as % conversion of corticosterone to 11-dehydrocorticosterone. Results are the means ± SEM, numbers of rats are given in parentheses. The rats were used from 6-8 litters in each experimental group. Significant values are shown *vs.* controls (CTR): $^*P<0.05$.

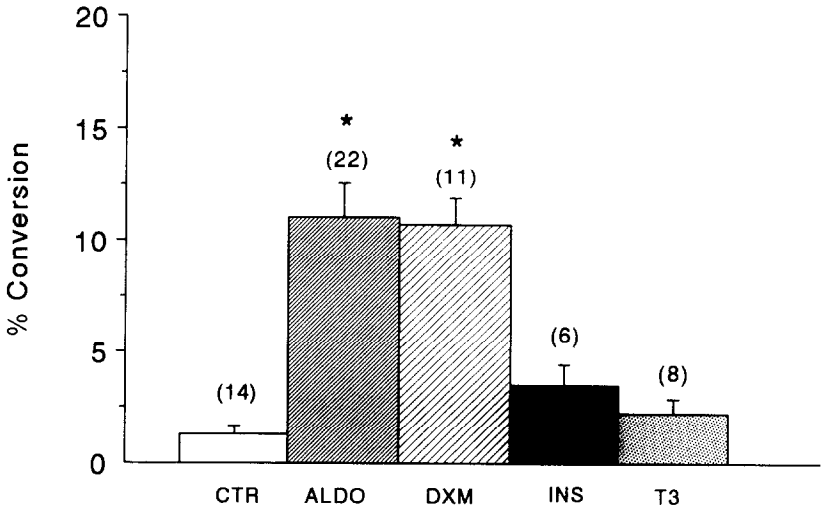

Fig. 2

Effect of corticosteroids, insulin, and triiodothyronine on 11βHSD activity in the ileum. Significant values are shown *vs.* controls (CTR): *P<0.01. For further details see Fig. 1.

corticosterone was much less expressed but the activity of 11βHSD in this segment was also significantly different from zero (P<0.01) (Fig.2). Preincubation of both intestinal segments with aldosterone or dexamethasone increased the activity of 11βHSD significantly even if this increase was much higher in the ileum than in the distal colon (Figs. 1,2). Aldosterone enhanced the activity by 768% in the ileum and only by 37% in the distal colon. The effect of dexamethasone was similar. Addition of triiodothyronine or insulin to the culture medium did not change corticosterone conversion to 11-dehydrocorticosterone except for ileum in which insulin had a marginal insignificant stimulatory effect on 11βHSD (Figs. 1,2).

To test whether the stimulation of corticosterone conversion in the explant cultures incubated in the presence of aldosterone (ALDO) or dexamethasone (DXM) can be inhibited by competitive inhibitors of 11βHSD, carbenoxolone (CBX) and progesterone (24), we performed additional series of experiments. Each intestinal segment was split longitudinally into two identical halves and one half was incubated with [^{3}H]corticosterone and the corresponding half was coincubated with CBX or progesterone. Progesterone completely inhibited the conversion of corticosterone to 11-dehydrocorticosterone in both segments. CBX was a little less potent inhibitor of this conversion both in the ileum and in the distal colon (TABLE 1). Although experiments with progesterone must be interpreted with caution because of its direct effect on mineralocorticoid receptors (25), the present data confirm that both the basal and induced conversion of [^{3}H]corticosterone in intestinal explants reflects the effect of 11βHSD.

Available evidence suggests that adrenalectomy decreases and glucocorticoid administration stimulates 11βHSD activity in aorta, hippocampus and intestine but not in the renal cortex (12, 15, 26, 27). Similarly, high-salt diet, which decreases the plasma concentration of aldosterone, can

TABLE 1

Inhibition of Stimulatory Effect of Corticosteroids on 11βHSD by Carbenoxolone in Explant Cultures of Ileum and Distal Colon

Treatment	Ileum	Distal colon
ALDO	9.6 ± 0.8 (8)	16.7 ± 1.4 (8)
ALDO + CBX	1.1 ± 0.3* (8)	1.6 ± 0.4* (7)
DXM	10.8 ± 1.0 (8)	15.2 ± 2.1 (8)
DXM + CBX	1.4 ± 0.4* (7)	1.5 ± 0.4* (8)

ALDO, aldosterone (10^{-7}M); CBX, carbenoxolone (5.10^{-6}M); DXM, dexamethasone (10^{-7}M). Numbers of rats (4 litters) are given in parentheses. Data are given in % conversion of corticosterone to 11-dehydrocorticosterone. Results are means ± SEM. Significant values are shown *vs.* controls (ALDO or DXM treated group): *$P<0.05$.

attenuate the activity of 11βHSD in immature intestine and the administration of deoxycorticosterone acetate to these salt-repleted rats prevents the decrease of the activity (12). In the present study, a direct effect of mineralocorticoids and glucocorticoids on ileal and colonic 11βHSD was demonstrated. The data indicate that both aldosterone and dexamethasone alter the activity of 11βHSD in explants of immature intestine. Since the circulating levels of both corticosterone and aldosterone increase progressively during the suckling and weaning period (17,18), the large increase of 11βHSD activity, which we have observed in developing ileum recently (12), may be due to elevated levels of corticosteroids.

In contrast, the explant cultures incubated with insulin or triiodothyronine did not show any changes of intestinal 11βHSD activity. Though some studies have demonstrated the effect of triiodothyronine and insulin on 11βHSD in fibroblasts and hepatocytes (13,14,22), our results do not indicate the possibility that thyroid hormones or insulin are active in developmental regulation of intestinal 11βHSD *via* a direct mechanism.

As both isoforms of dehydrogenase, 11βHSD1 and 11βHSD2, are present in the adult colon (28) we are not able to conclude which isoform is regulated by corticosteroids. However, we have used a substrate concentration 13 nM which is similar to K_m of colonic 11βHSD2 (≈50 nM) and not 11βHSD1 (≈1 μM) (28) and thus we assume that our data reflect the effect of corticosteroids on 11βHSD2. The mechanism of the corticosteroid induction of 11βHSD remains to be determined.

Acknowledgements

This work was supported by grant no. 305/94/1719 from the Grant Agency of the Czech Republic.

References

1. J.W. FUNDER, P.T. PEARCE, R. SMITH and A.I. SMITH, Science 242 583-585 (1988).
2. C.R.W. EDWARDS and P.M. STEWART, J. Steroid Biochem. Mol. Biol. 39 859-865 (1991).
3. V. LAKSHMI and C. MONDER, Endocrinology 123 2390-2398 (1988).
4. A.K. AGARWAL, C. MONDER, B. ECKSTEIN and P.C. WHITE, J. Biol. Chem. 264 18939-18943 (1989).
5. E. RUSVAI and A.NARAY-FEJES-TOTH, J. Biol. Chem. 268 10717-10720 (1993).
6. K. YANG, J. Steroid. Biochem. Mol. Biol. 52 337-343 (1995).
7. A.K. AGARWAL, T. MUNE, C. MONDER and P.C. WHITE, J. Biol. Chem. 269 25959-25962 (1994).
8. Z. KROZOWSKI, A.L. ALBISTON, V.R. OBEYESEKERE, R.K. ANDREWS and R.E. SMITH, J. Steroid Biochem. Mol. Biol. 55 457-464 (1995).
9. P.M. STEWART, C.B. WHORWOOD and J.I. MASON, J. Steroid Biochem. Mol. Biol. 55 465-471 (1995).
10. C. LECKIE, K.E. CHAPMAN, C.R.W. EDWARDS and J.R. SECKL, Endocrinology 136 5561-5569 (1995).
11. M.P. MOISAN, C.R.W. EDWARDS and J.R. SECKL, Endocrinology 130 400-404 (1992).
12. J. PÁCHA and I. MIKŠÍK, J. Endocrinol. 148 561-566 (1996).
13. M.M. HAMMAMI and P.K. SIITERI, J. Clin. Endocrinol. Metab. 73 326-334 (1991).
14. C.B. WHORWOOD, M.C. SHEPPARD and P.M. STEWART, J. Steroid Biochem. Mol. Biol. 46 539-547 (1993).
15. B.R. WALKER, B.C. WILLIAMS and C.R.W. EDWARDS, J. Endocrinol. 141 467-472 (1994).
16. A.L. ALBISTON, R.E. SMITH and Z.S. KROZOWSKI, Mol. Cell. Endocrinol. 109 183-188 (1995).
17. S. HENNING, Am. J. Physiol. 235 E451-E456 (1978).
18. J. PÁCHA, I. POHLOVÁ and P. KAREN, Pediatr. Res. 38 356-360 (1995).
19. P. WALKER, J.D. DUBOIS and J.H. DUSSAULT, Pediatr. Res. 14 247-249 (1980).
20. E. BLAZGUEZ, E. MONTOYA and C.L. QUIJADA, J. Endocrinol. 48 553-561 (1970).
21. J. KOLÍNSKÁ, M. BAUDYŠOVÁ, M. ZÁKOSTELECKÁ, J. KRAML and L. KADLECOVÁ, Biochem. Int. 22 495-508 (1990).
22. Y. LIU, Y. NAKAGAWA, K. NASUDA, H. SAEGUSA and Y. IGARASHI, Life Sci. 59 227-234 (1996).
23. H.-P. GAEGGELER, H. DUPERREX, S. HAUTIER and B.C. ROSSIER, Am. J. Physiol. 264 C317-C322 (1993).
24. H. MORITA, M. ZHOU, M. FOECKING, E.P. GOMEZ-SANCHEZ, E.N. COZZA and C.E. GOMEZ-SANCHEZ, Endocrinology 137 2308-2314 (1996).
25. R. RUPPRECHT, J.M.H.M. REUL, B. van STEENSEL, D. SPENGLER, M. SÖDER, B. BERNING, F. HOLSBOER and K. DAMM, Eur. J. Pharmacol. 247 145-154 (1993).
26. R.E. SMITH and J.W. FUNDER, J. Steroid Biochem. Mol. Biol. 38 265-267 (1991).
27. J. PÁCHA and I. MIKŠÍK, Life Sci. 54 745-749 (1994).
28. C.B. WHORWOOD, M.L. RICKETTS and P.M. STEWART, Endocrinology 135 2523-2541 (1994).