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Glucocorticoid Availability in Colonic Inflammation of Rat

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Abstract Recent in vitro studies have shown the
involvement of pro-inflammatory cytokines in the regulation
of the local metabolism of glucocorticoids via 11β-hydroxy-
steroid dehydrogenase type 1 and type 2 (11HSD1 and
11HSD2). However, direct in vivo evidence for a relation-
ship among the local metabolism of glucocorticoids,
inflammation and steroid enzymes is still lacking. We have
therefore examined the changes in the local metabolism of
glucocorticoids during colonic inflammation induced by
TNBS and the consequences of corticosterone metabolism
inhibition by carbenoxolone on 11HSD1, 11HSD2, cyclo-
oxygenase 2 (COX-2), mucin 2 (MUC-2), tumor necrosis
factor α (TNF-α), and interleukin 1β (IL-1β). The metabo-
lism of glucocorticoids was measured in tissue slices in vitro
and their 11HSD1, 11HSD2, COX-2, MUC-2, TNF-α,
and IL-1β mRNA abundances by quantitative reverse
transcription-polymerase chain reaction. Colitis produced an
up-regulation of colonic 11HSD1 and down-regulation of
11HSD2 in a dose-dependent manner, and these changes
resulted in a decreased capacity of the inflamed tissue to
inactivate tissue corticosterone. Similarly, 11HSD1 tran-
script was increased in colonic intraepithelial lymphocytes
of TNBS-treated rats. Topical intracolonic application of

carbenoxolone stimulated 11HSD1 mRNA and partially
inhibited 11HSD2 mRNA and tissue corticosterone inacti-
vation and these changes were blocked by RU-486. The
administration of budesonide mimicked the effect of car-
benoxolone. In contrast to the local metabolism of
glucocorticoids, carbenoxolone neither potentiates nor
diminishes gene expression for COX-2, TNF-α, and IL-1β,
despite the fact that budesonide down-regulated all of them.
These data indicate that inflammation is associated with the
down-regulation of tissue glucocorticoid catabolism. How-
ever, these changes in the local metabolism of
glucocorticoids do not modulate the expression of COX-2,
TNF-α, and IL-1β in inflamed tissue.

Keywords 11β-hydroxysteroid dehydrogenase ·
Inflammation · TNBS-colitis

Introduction

Glucocorticoids are hormones that regulate a variety of
homeostatic processes including metabolism, cell prolif-
eration and differentiation, and immune functions
including inflammation. Acute inflammatory response is
associated with an increase in glucocorticoid levels via
the stimulation of pro-inflammatory cytokines and activa-
tion of the hypothalamo–pituitary–adrenal axis [1].
Although the immunosuppressive role of glucocorticoids is
well established, there is accumulating evidence that
glucocorticoids may not only suppress, but also enhance
immune functions within a physiological concentration
range [2]. One mechanism by which this may be
achieved is via the exposure of immune cells to higher
local concentrations of glucocorticoids. Within target
cells or tissues the glucocorticoid action depends not only

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on the plasma level of the hormone, its receptors and receptor-effector coupling, but also on the local metabolism of glucocorticoids. Endogenous glucocorticoids exist either as active 11-hydroxy steroids (cortisol and corticosterone) that bind glucocorticoid receptors or as inactive 11-oxo derivatives (cortisone and 11-dehydrocorticosterone), which do not. The local regulation of glucocorticoid availability is determined by the prereceptor interconversion of active and inactive ligands mediated by 11β-hydroxysteroid dehydrogenase. Two distinct types of this enzyme have been cloned and characterized. Type 1 (11HSD1) is a $NADP^+(H)$-dependent enzyme whose reductase activity predominates in intact cells. This enzyme activates cortisol and corticosterone from their 11-keto derivatives and thus increases the local concentration of active glucocorticoid. In contrast, type 2 (11HSD2) requires NAD^+ as a co-substrate and possesses only dehydrogenase activity, thereby inactivating endogenous glucocorticoid hormones [3, 4].

From this point of view, one could expect that the bidirectional shuttle of glucocorticoids is of high importance in inflammation because the production of active ligands by the up-regulation of 11HSD1 and/or down-regulation of 11HSD2 might counteract the inflammatory response. It recently has been shown that the inhibition of 11HSDs with carbenoxolone potentiates the anti-inflammatory effect of glucocorticoids in mesangial and airway epithelial cells [5–8]. Furthermore, pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor α (TNF-α) stimulate 11HSD1 and down-regulate 11HSD2 [5, 9–13]. These observations indicate that pro-inflammatory cytokines are able to modulate intracellular corticosterone metabolism, and thus they might be able to counterbalance their own pro-inflammatory effect.

The interactions between the local metabolism of glucocorticoids and pro-inflammatory cytokines have been studied in experiments in vitro, and their interactions in vivo have yet to be defined. Moreover, some data indicate that the local metabolism of glucocorticoids can be modulated not only by cytokines, but also by glucocorticoids themselves [4, 7, 14]. As the relationship between locally produced glucocorticoids and inflammation has received only limited attention we examined the influence of modulation of the local metabolism of glucocorticoids by carbenoxolone on the expression of HSD1 and HSD2 and the pro-inflammatory cytokines, cyclooxygenase 2 (COX-2), myeloperoxidase (MPO) and mucin 2 (MUC-2) in inflamed rat colon that showed up-regulation of 11HSD1 and down-regulation of 11HSD2 [15]. The efficacy of carbenoxolone was compared with budesonide, a reference glucocorticoid drug used in the treatment of colitis.

Methods

Animals

Male Wistar rats (Institute of Physiology, Czech Academy of Science, Prague) weighing 250–300 g were maintained at 22°C and a constant photoperiod (12 h light, 12 h dark). Water and standard laboratory feed were available ad libitum. Colitis was induced using the technique of Morris et al. [16] by a single enema of the hapten 2,4,6-trinitrobenzenesulphonic acid, TNBS (Fluka, Buchs, Switzerland). TNBS in 50% ethanol was infused 8 cm into the colon via the anus, and the dose of TNBS was the commonly used 30 mg dose; in some experiments a lower dose of 8 mg was used in order to reduce the severity of the inflammatory response. Control animals received physiological saline instead of TNBS solution in ethanol. The TNBS-treated rats were randomly divided into four groups with different treatments that started 24 h after colitis induction and lasted 5 days. The first group received a saline enema, the second group a 4 mg dose of carbenoxolone, the inhibitor of 11HSDs, the third group was treated with a 4 mg dose of carbenoxolone in together with 1 mg RU-486, an antagonist of glucocorticoid receptors, and the last group received a 200 µg dose of budesonide. This synthetic glucocorticoid was used because it is thought to be not metabolized by 11HSDs [17]. The drugs were administered once daily into the colon. All rats were killed on day 7 after TNBS administration, and sections of the inflamed colon were collected for further analysis. The experiments were approved by the Institute of Physiology Animal Ethics Committee.

Intraepithelial lymphocyte (IEL) preparation

IELs were obtained as originally described by Lyscom and Brueton [18] with some modifications. Briefly, the inflamed part of the colon was dissected and the lumen gently flushed with 40 ml of cold physiological saline (4°C). The intestine was then incised longitudinally and cut into 5-mm pieces. The pieces were incubated in 50 ml flasks containing 40 ml of RPMI-1640 medium supplemented with fetal bovine serum (12.5%) for 20 min at 37°C with stirring. The incubation step was repeated thrice, the supernatants were combined, and IELs were separated from epithelial cells by discontinuous Percoll gradient at the interface 67%/44%. The harvested cells were washed in RPMI medium and used for further analysis.

11β-oxidase and 11-reductase activities in intact tissue slices

The 11β-oxidase (dehydrogenase) and 11-reductase activities of 11βHSD were measured ex vivo in fresh slices of colonic tissue as mentioned earlier [19]. Colonic sections were cut with a razor blade into small strips 1–2 mm wide and incubated for 80 min in sealed vessels containing 250 mg of tissue wet weight and an oxygenated incubation solution (in mM: NaCl, 119.0; CaCl$_2$, 1.2; MgCl$_2$, 1.2; NaHCO$_3$, 21.0; K$_2$HPO$_4$, 2.4; KH$_2$PO$_4$, 0.6; glucose, 10.0; glutamine, 2.5; β-hydroxybutyrate, 0.5, mannitol, 10.0; 37°C) and 1.45 μM corticosterone (11β-oxidase assay) or 1.45 μM 11-dehydrocorticosterone (11-reductase assay). At the end of incubation, an internal standard of deoxycorticosterone (1.45 μM) was added, and the solution was chilled on ice, extracted and analyzed using HPLC as described earlier [20].

Quantitative analysis of 11HSD1, 11HSD2, TNF-α, IL-1β, COX-2, and MUC-2 mRNAs

Total RNA was extracted from an inflamed part of the tissue using Trizol (Invitrogen), diluted for optimal concentration (0.5–1.0 μg/μl), and RNA was reverse transcribed using random primers and Moloney murine leukemia virus reverse transcriptase (Invitrogen) according to the manufacturer's protocol. The cDNA was analyzed by real-time RT-PCR using LightCycler (colonic 11HSD1 and 11HSD2) or TaqMan technology (TNF-α, IL-1β, COX-2, and MUC-2 in colon and 11HSD1 in IELs). The target mRNA was quantified in relation to the abundance of β-actin (LightCycler experiments) or glyceraldehyde-3-phosphate dehydrogenase, GAPD (TaqMan experiments).

The relative mRNA levels of colonic 11HSD1, 11HSD2, and β-actin mRNAs were analyzed as described previously [15]. Briefly, a 1-μl aliquot of the ten-fold diluted RT reaction product was subjected to PCR using a LightCycler instrument (Roche Diagnostics GmbH, Mannheim, Germany); LightCycler-DNA Master Sybr Green I mix; primers, 0.5 μM, MgCl$_2$, 3 mM (11HSD1), 4 mM (11HSD2), or 5 mM (β-actin). The product purity was verified by melting analysis. The amplification curves of unknown samples were compared with those of the serial dilutions of standard cDNA from the colon, and the amounts of 11HSD1 and 11HSD2 were calculated using standard calibration curves as the proportionate quantity of both isoforms of 11HSD relative to β-actin. The sequences of primers were as follows (5′ > 3′): 11HSD1, sense GAGTTCAGACCAGAAATGCTCC, antisense TGTGTGATGTGATTGAGAATGAGC; 11HSD2, sense GATGTTCCCCTCGCCTGAA, antisense ATGAGCAGTGCAATAG

CTGCCTTG; β-actin, sense CCGTAAAGACCTCTATGCCA, antisense AAGAAAGGGTGTAAAACGCA.

The mRNA levels of TNF-α, IL-1β, COX-2, and MUC-2 in colon, 11HSD1 in IELs and internal control GAPD were analyzed using TaqMan quantitative real-time PCR. The RT product was diluted ten-fold and then subjected to PCR using Abi-Prism 7,000 Sequence Detection System instrument (Applied Biosystems), TaqMan Universal PCR Master Mix with AmpEraseUNG (Applied Biosystems) and pre-made TaqMan Gene Expression Assays for COX-2 (cat. no. Rn00568225 -m1), 11β-HSD1 (cat. no. Rn00567167-m1), TNF-α (cat. no. Rn00562055-m1), IL-1β (cat. no. Rn015-14151-m1), MUC-2 (Rn01498195-m1) or TaqMan Endogenous Control Rat GAPD (VIC/MGB) (cat. no. 4352338) in a final volume 20 μl. The thermal cycler conditions were: UNG activation for 2 min at 50°C followed by 95°C for 10 min, subsequent amplification consisted of 40 cycles of denaturation for 15 s and annealing and extension at 60°C for 1 min. Levels of mRNA were calculated from the standard curves generated for each run and the curves were constructed with serial dilutions of standard cDNA.

Assay of MPO

MPO activity was assessed as an index of neutrophil infiltration using the method of Bradley et al. [21] in full thickness specimens that had been taken from the colon and stored at −70°C. One unit of enzyme activity was defined as that degradating 1 μmol of hydrogen peroxide per minute at 22°C.

Statistical analysis

All data are expressed as mean ± SEM. Statistical comparisons were made between drug-treated groups using a one-way ANOVA followed by a Newman–Keuls comparison post hoc test and between IEL of control and TNBS-treated rats by Student's t-test using the package Statistica v.6 (StatSoft Inc., Tulsa, OK). Statistical significance was assessed at $P < 0.05$.

Results

The administration of TNBS/ethanol resulted in significant changes of 11β-dehydrogenase and 11-oxoreductase activities in colonic tissue, and these changes depended on the dose of TNBS. In untreated control animals, freshly prepared slices of colonic tissue oxidized corticosterone to 11-dehydrocorticosterone (51.4 ± 5.6 ng/h.mg DW) and reduced 11-dehydrocorticosterone to corticosterone

$(2.7 \pm 0.4$ ng/h.mg DW). As shown in Fig. 1, 11β-dehydrogenase activity was reduced in a dose-dependent manner in rats treated with TNBS. In contrast, 11-reductase was strongly up-regulated in inflamed tissue; nevertheless, this stimulation was not high enough to reverse the net activity of 11HSDs (11-oxidation − 11-reduction). This net activity in all three groups was of oxidation of corticosterone to 11-dehydrocorticosterone, but it was higher in the healthy colon (48.7 ± 6.3 ng/h.mg DW) than in rats treated with 8 mg or 30 mg TNBS (37.2 ± 3.7 and 10.0 ± 2.0 ng/h.mg DW). The MPO activity in healthy colon was 0.7 ± 0.1 U/g tissue, whereas in inflamed tissue the MPO activity reached significantly higher values: 2.6 ± 0.8 U/g tissue in rats that had received 8 mg TNBS and 4.0 ± 1.1 U/mg tissue in animals with 30 mg TNBS. The novel finding of 11HSD1 stimulation during activation of lymphocytes [22] led us to the question of whether the
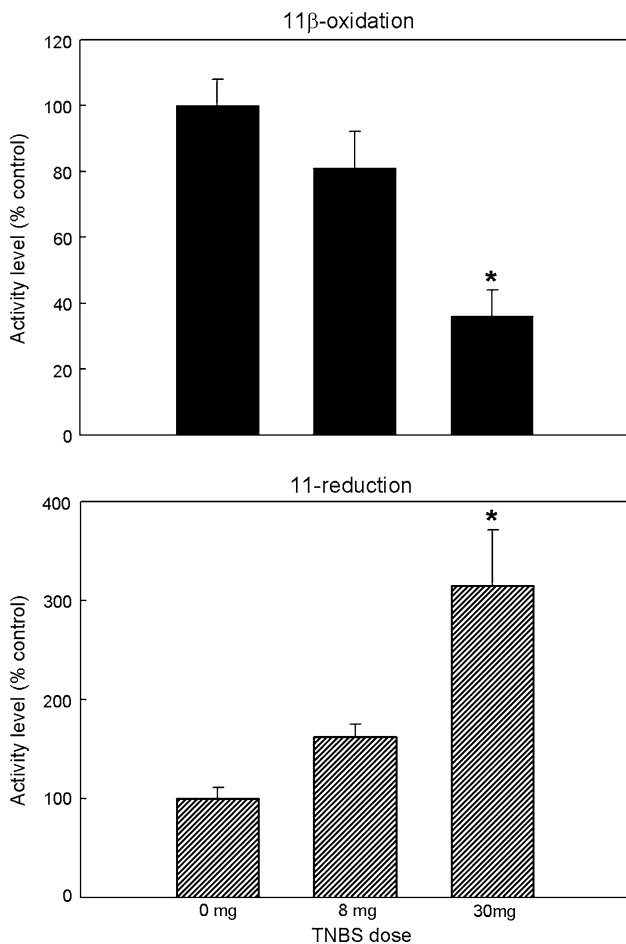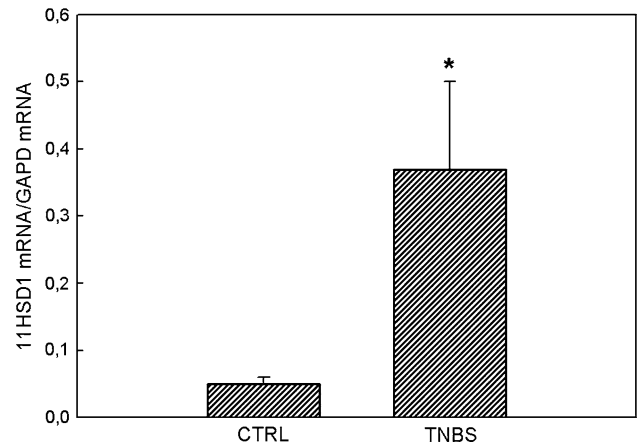
Fig. 2 Expression of 11HSD1 mRNA in colonic intraepithelial lympocytes of rats with colitis and in healthy controls. The data are expressed as mean $\pm$ SEM of six rats/group. *$P < 0.05$ compared with control group

up-regulation of 11HSD1 in inflamed tissue reflects activation of colonic immune cells. As shown in Fig. 2, the IELs from inflamed colon dramatically increased the level of 11HSD1 mRNA.

When the TNBS rats were topically treated with carbenoxolone, corticosterone oxidation was decreased in the inflamed tissue by almost 50% (Fig. 3). The co-administration of carbenoxolone with the glucocorticoid antagonist
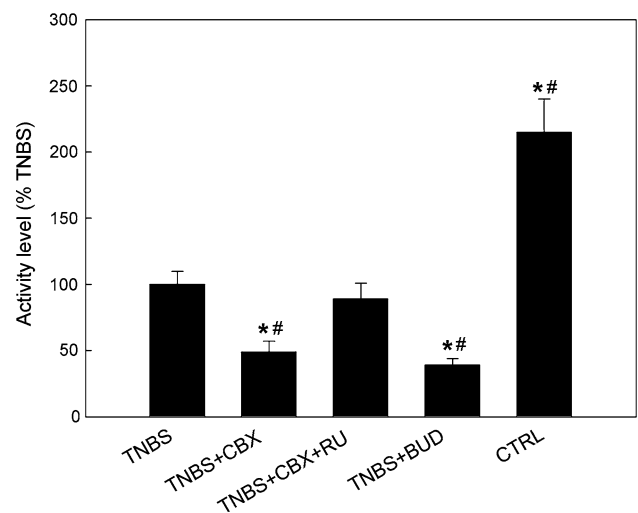
Fig. 1 Effect of various doses of TNBS on the colonic metabolism of glucocorticoids. 11β-dehydrogenase (11HSD2) and 11-reductase (11HSD1) activities were measured in tissue slices of rats exposed to 8 mg or 30 mg TNBS on day 7 after colitis induction. The data are shown as a percentage of control untreated rats and is expressed as mean $\pm$ SEM of eight rats/group. *$P < 0.05$ compared with control group

Fig. 3 Metabolism of corticosterone in inflamed colonic tissue treated with carbenoxolone (CBX), mifepristone (RU-486), and budesonide (BUD). Conversion of corticosterone to 11-dehydrocorticosterone was measured in colonic slices of rats exposed to 30 mg TNBS followed by daily topical intracolonic administration of CBX, RU-486, BUD, or physiological saline for 5 days; CTRL, untreated controls. The data are shown as a percentage of TNBS-treated rats with intracolonic administration of saline and are expressed as mean $\pm$ SEM of 16–24 rats/group. *$P < 0.05$ compared with TNBS group, #$P < 0.05$ compared with TNBS + CBX + RU group

RU-486 attenuated the effect of carbenoxolone. To test the possibility that the effect of carbenoxolone reflects a glucocorticoid "self-regulating" effect, identical experiments were performed with budesonide. As shown in Fig. 3, budesonide decreased the ability of the inflamed tissue to inactivate corticosterone similarly to carbenoxolone.

To assess the impact of the tested drugs on 11HSD1 and 11HSD2, we measured the transcript levels of both isoforms. As shown in Fig. 4a, the treatment of TNBS rats with carbenoxolone up-regulated 11HSD1 mRNA, and this effect was completely inhibited by RU-486. These data suggest that colonic 11HSD1 may be responsive to glucocorticoids, because identical results were found in experiments with budesonide (Fig. 4a). In contrast to 11HSD1, budesonide had no significant effect on 11HSD2 mRNA, whereas carbenoxolone decreased 11HSD2 mRNA expression, and this effect was attenuated in the presence of RU-486 (Fig. 4b).
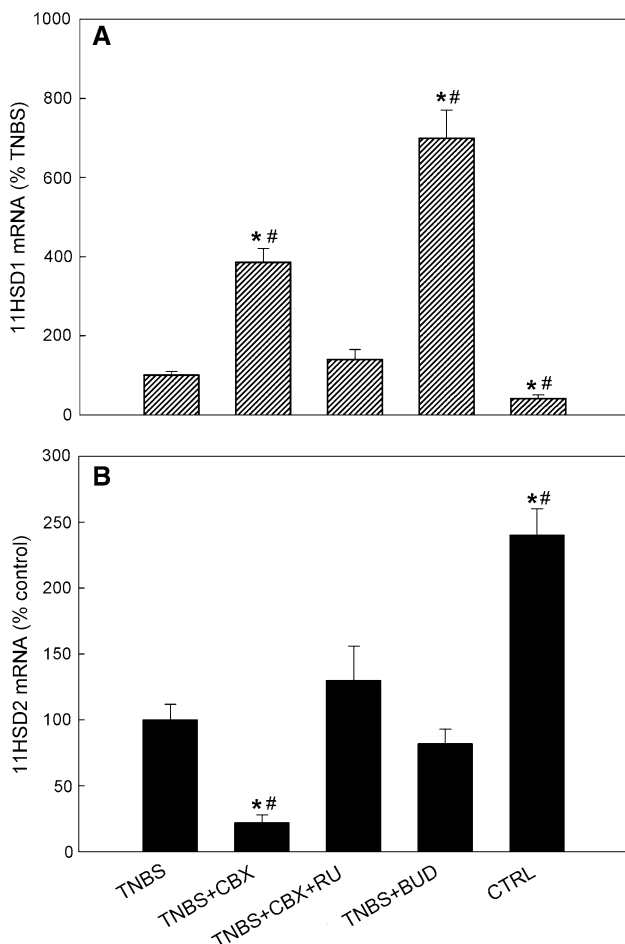
The rat colons were also examined for MPO activity and the expression of TNF-α, IL-1β, MUC-2, and COX-2 mRNAs. MPO activity in the colon of TNBS rats was significantly increased after treatment with RU-486 and decreased after treatment with budesonide; however there was no significant effect on MPO activity with carbenoxolone (Table 1). Similar changes were found with pro-inflammatory cytokines. Budesonide down-regulated TNF-α mRNA and IL-1β mRNA, whereas RU-486 up-regulated their expression. In contrast, carbenoxolone had no apparent effect. The transcript levels of COX-2 were only significantly down-regulated after budesonide; neither carbenoxolone nor RU-486 significantly modulated the level of COX-2 expression. In contrast, budesonide was without any effect on expression of MUC-2, and carbenoxolone stimulated its expression in both the presence and absence of RU-486.

Discussion

TNBS-induced colitis is a common animal model of inflammation that simulates Crohn's disease relatively well due to the discontinuous damage associated with the transmural infiltration of immune cells [16]. The inflammation is predominantly mediated by activating pro-inflammatory cytokines and enzymes such as TNF-α, IL-1β, COX-2, and inducible nitric oxide synthase via activating the NF-κB pathway [23–25] and accompanied with up-regulation of mucin production via pro-inflammatory cytokines and prostaglandins [26]. A substantial amount of data shows that corticosteroids are powerful drugs that down-regulate inflammation in various tissues including colitis by modulating various immune pathways such as the NF-κB pathway and pathways synthesizing cytokines and prostaglandins [27]. Because TNF-α and IL-1β have been shown to up-regulate 11HSD1 and down-regulate 11HSD2 in cell cultures [5, 9–13], these cytokines could either diminish or increase the local concentration of glucocorticoids in inflamed tissue, and a high ratio of 11-dehydrocorticosterone reduction to 11β-corticosterone oxidation might generate a negative feedback loop suppressing further inflammatory response as was proposed by Escher et al. [5].

The finding of decreased 11HSD2 activity in inflamed tissue [15, 28] led us to the hypothesis that the local metabolism of glucocorticoids could modulate the inflammation-associated gene expression during colitis. To test this hypothesis, we treated animals with carbenoxolone and measured the metabolism of corticosterone (11HSD1 and 11HSD2), the markers of colonic inflammation (TNF-α, IL-1β, COX-2 and MPO), and the most prominent mucin secreted by intestinal goblet cells (MUC-2). Since 11HSD2 is only expressed in enterocytes, whereas 11HSD1 is

Fig. 4 Effect of carbenoxolone (CBX), mifepristone (RU-486), and budesonide (BUD) on expression of 11HSD1 mRNA (**a**) and 11HSD2 mRNA (**b**) in colon of TNBS-treated rats. For further details see Fig. 3. Each column represents mean ± SEM of eight to ten rats/group. *$P < 0.05$ compared with TNBS group, #$P < 0.05$ compared with TNBS + CBX + RU group

Table 1 Effect of carbenoxolone, RU-486, budesonide, and physiological saline treatment on myeloperoxidase activity (MPO), and on expression of pro-inflammatory cytokines TNF-α mRNA, and IL-1β mRNA, cyclooxygenase-2 (COX-2) mRNA, and mucin 2 (MUC-2) mRNA in colon of TNBS-treated rats

	TNBS	TNBS + carbenoxolone	TNBS + RU 486 + carbenoxolone	TNBS + budesonide	Control
MPO activity	4.5 ± 0.5[#]	4.2 ± 0.6[#]	7.1 ± 0.7[*]	2.2 ± 0.4[*,#]	1.9 ± 0.3[*,#]
TNF-α mRNA	1.0 ± 0.1[#]	0.8 ± 0.1[#]	2.1 ± 0.5[*]	0.3 ± 0.1[*,#]	0.5 ± 0.1[*,#]
IL-1β mRNA	0.8 ± 0.2	0.7 ± 0.1	1.4 ± 0.3	0.3 ± 0.1[*,#]	0.4 ± 0.2[#]
COX-2 mRNA	3.5 ± 0.5	3.3 ± 0.5	3.1 ± 0.6	0.6 ± 0.1[*,#]	0.4 ± 0.1[*,#]
MUC-2 mRNA	1.1 ± 0.4[#]	3.0 ± 0.4[*]	3.3 ± 0.4[*]	1.3 ± 0.3[#]	0.9 ± 0.2[#]

CTRL, untreated rats; myeloperoxidase activity is given in U/g tissue, and cytokine, MUC-2 and COX-2 expression in arbitrary units relative to those of GAPD. Data are expressed as mean ± SEM of eight to ten animals in each group. * $P < 0.05$ compared with TNBS group, # $P < 0.05$ compared with TNBS + RU + CBX group

expressed in the cells of lamina propria such as fibroblasts and immune cells [22, 29, 30], it can be assumed that inhibition of 11HSD2 facilitates the access of corticosterone to the glucocorticoid receptors in some cells, whereas inhibition of 11HSD1 decreases the conversion of inactive 11-dehydrocorticosterone to corticosterone in others and thus restricts the access of corticosterone to its receptor. Our data show that local administration of carbenoxolone causes a decrease in 11β-dehydrogenase bioactivity in colonic tissue and that this decrease is accompanied by an up-regulation of 11HSD1 mRNA expression and down-regulation of 11HSD2 mRNA expression. The decreased metabolism of corticosterone in inflamed tissue does not seem to reflect the presence and direct effect of carbenoxolone in the tissue during assay (24 h after last enema, interaction with RU-486), but is most likely due to an increased local activity of 11HSD1 and decreased activity of 11HSD2. Based on these findings, we can summarize that carbenoxolone alone or the changes in local concentration of corticosterone during carbenoxolone treatment are able to up-regulate 11HSD1 and down-regulate 11HSD2.

11HSD1 has been shown to be up-regulated by glucocorticoids in several cell types [4], and this induction has been blocked by RU-486 [31]. In contrast to 11HSD1, the effect of carbenoxolone on 11HSD2 mRNA expression cannot be adequately explained by "self-regulation" of this enzyme via its substrate or product because glucocorticoids appear to up-regulate 11HSD2 [7, 14], and thus the effect of other factors cannot be excluded. The simplest interpretation of this finding is that derivatives of arachidonic acid might play a role in the regulation of colonic 11HSD2. Locally produced prostaglandins decrease 11HSD2 activity [32], and the major enzyme of prostaglandin inactivation, 15-hydroxy-PG-dehydrogenase, is reduced by glucocorticoids [33] and inhibited by carbenoxolone [34]. The effect of RU-486 that acted as an agonist stimulating 11HSD2 cannot be explained in the context of our experiments; however, it is noteworthy that the similar stimulatory effect

of RU-486 has been shown previously in Ishikawa cells [35].

Glucocorticoids via glucocorticoid receptors have been previously demonstrated to reduce inflammation through the inhibition of phospholipase A2 and COX-2 [36] and through the modulation of cytokine production [26]. Considering that carbenoxolone administration modulates local concentration of corticosterone, it is surprising that carbenoxolone has no effect on the expression of COX-2, TNF-α and IL-1β. This finding contrasts with the results of experiments with budesonide. Our interpretation of these findings is that under inflammatory circumstances the local metabolism of corticosterone, as a modulator of glucocorticoid hormone action, is unimportant in the regulation of gene expression for COX-2, TNF-α, and IL-1β. Nevertheless, their expression is regulated by glucocorticoids as shown in our experiments with budesonide and in the paper of Nakase et al. [37] using orally administered dexamethasone. The data for RU-486 indicate that systemic corticosterone is more important in the regulation of some pro-inflammatory cytokines and MPO than local changes. The study also suggests that the local metabolism of corticosterone is not able to influence the infiltration and activation of granulocytes into colonic tissue. The activity of MPO, which correlates well with the infiltration of tissue by granulocytes [38], was decreased after treatment with budesonide, but not with carbenoxolone. Assuming that glucocorticoids decrease MPO activity [37] and the influx of inflammatory cells into the colon of TNBS rats [39], it is obvious that the changes in the local metabolism of corticosterone are not able to modulate granulocyte migration and activation. In contrast to our expectation, neither carbenoxolone nor budesonide suppressed MUC-2 expression even if colitis modulates MUC gene expression [40], and experiments performed with intestinal mucus-secreting cell lines showed modulation of transcript levels for various mucins by IL-1 and corticosteroids [41, 42].

The most significant finding of the study presented here is that colonic inflammation up-regulates 11HSD1,

depending on the extent of the inflammation, and that the changes in 11HSD1 and 11HSD2 are associated with a decreased ability of the inflamed tissue to oxidase biologically active glucocorticoids to their inactive 11-oxo derivatives. As 11HSD1 is expressed in the intestinal compartment of lamina propria and gut-associated lymphatic tissue [29] and TNBS colitis is associated with massive intramural infiltration of immune cells [43] that express 11HSD1 [22], we suppose that up-regulation of 11HSD1 mRNA and 11-reductase activity during colitis may reflect the increased colonic infiltration with immune cells and the stimulation of 11HSD1 by proinflammatory mediators. Considering that TNF-α and IL-1β are increased in the colon of rats with TNBS colitis [44] and these cytokines have been shown to stimulate 11HSD1 and decrease 11HSD2 in cell culture experiments [5, 9–13], our results are in agreement with the regulatory role of proinflammatory cytokines in the glucocorticoid metabolism of inflamed colon. Furthermore, the effect of carbenoxolone and its inhibition by RU-486 suggest a "self-regulating" effect of corticosterone on the up-regulation of colonic 11HSD1. On the other hand, our study indicates that the corticosterone regulatory pathways controlling proinflammatory processes are not closely regulated by 11HSDs during fully developed acute colitis. In contrast to the results of in vitro experiments [5, 11], our experiments are not consistent with the hypothesis that the negative feedback between the local metabolism of glucocorticoids and pro-inflammatory processes may serve any physiological role in fully developed acute inflammation. Whether the local metabolism of glucocorticoids might play a role during the early phases of colonic inflammation remains to be seen.

References

1. Besedovsky HO, del Rey A (1996) Immune-neuro-endocrine interactions: facts and hypotheses. Endocr Rev 17:64–102
2. Wilckens T, De Rijk R (1997) Glucocorticoids and immune function: unknown dimensions and new frontier. Immunol Today 18:418–424
3. Stewart PM, Krozowski ZS (1999) 11β-hydroxysteroid dehydrogenase. Vit Horm 57:249–324
4. Tomlinson JW, Walker EA, Bujalska IJ, Draper N, Laverty GG, Cooper MS, Hewison M, Stewart PM (2004) 11β-hydroxysteroid dehydrogenase type 1: a tissue specific regulator of glucocorticoid response. Endocr Rev 25:831–866
5. Escher G, Galli I, Vishwanath BS, Frey BM, Frey FJ (1997) Tumor necrosis factor α and interleukin 1β enhance the corticone/cortisol shuttle. J Exp Med 186:189–198
6. Feinstein MB, Schleimer RP (1999) Regulation of the action of hydrocorticone in airway epithelial cells by 11β-hydroxysteroid dehydrogenase. Am J Respir Cell Mol Biol 21:403–408
7. Suzuki S, Koyama K, Darnel A, Ishibashi H, Kobayashi S, Kubo H, Suzuki T, Sasano H, Krozowski ZS (2003) Dexamethasone upregulates 11β-hydroxysteroid dehydrogenase type 2 in BEAS-2B cells. Am J Respir Crit Care Med 167:1244–1249
8. Suzuki S, Matsuda Y, Sugawara T, Tabata T, Ishibashi H, Hoshikawa Y, Kubo H, Kondo T (2004) Effects of carbenoxolone on alveolar fluid clearance and lung inflammation in the rat. Crit Care Med 32:1910–1915
9. Tomlinson JW, Moore J, Cooper MS, Bujalska I, Shahmanesh M, Burt C, Strain A, Hewison M, Stewart PM (2001) Regulation of expression of 11β-hydroxysteroid dehydrogenase type 1 in adipose tissue: tissue-specific induction by cytokines. Endocrinology 142:1982–1989
10. Heiniger CD, Rochat MK, Frey FJ, Frey BM (2001) TNF-α enhances intracellular glucocorticoid availability. FEBS Lett 507:351–356
11. Cooper MS, Bujalska I, Rabbitt E, Walker EA, Bland R, Sheppard MC, Hewison M, Stewart PM (2001) Modulation of 11β-hydroxysteroid dehydrogenase isozymes by proinflammatory cytokines in osteoblasts: an autocrine switch from glucocorticoid inactivation to activation. J Bone Miner Res 16:1037–1044
12. Yong PYK, Harlow C, Thong CJ, Hillier SG (2002) Regulation of 11β-hydroxysteroid dehydrogenase type 1 gene expression in human ovarian surface epithelial cells by interleukin-1. Human Reprod 9:2300–2306
13. Suzuki S, Tsubochi H, Ishibashi H, Matsuda Y, Suzuki T, Krozowski ZS, Sasano H, Kondo T (2005) Inflammatory mediators down-regulate 11β-hydroxysteroid dehydrogenase type 2 in a human lung epithelial cell line BEAS-2B and rat lung. Tohoku J Exp Med 207:293–301
14. Pácha J, Mikšík I, Lisá V, Pohlová I (1997) Hormonal regulation of intestinal 11β-hydroxysteroid dehydrogenase. Life Sci 61:2391–2396
15. Bryndová J, Žbánková Š, Kment M, Pácha J (2004) Colitis up-regulates local glucocorticoid activation and down-regulates inactivation in colonic tissue. Scand J Gastroenterol 39:549–553
16. Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL (1989) Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology 96:795–803
17. Erdbäcker S, Andersson P, Lindberg C, Paulson J, Ryrfeldt A, Thalen A (1987) Liver metabolism of budesonide in rat, mouse, and man. comparative aspects. Drug Metab Dispos 15:403–411
18. Lyscom N, Brueton MJ (1982) Intraepithelial, lamina propria and peyer's patch lymphocytes of the rat small intestine: isolation and characterization in terms of immunoglobulin markers and receptors for monoclonal antibodies. Immunology 45:775–783
19. Pácha J, Mikšík I (1996) 11β-hydroxysteroid dehydrogenase in developing rat intestine. J Endocrinol 148:561–566
20. Pácha J, Mikšík I, Mrnka L, Zemanová Z, Bryndová J, Mazancová K, Kučka M (2004) Corticosteroid regulation of colonic ion transport during postnatal development: methods for corticosterone analysis. Physiol Res 53:S63-S80
21. Bradley PP, Priebat DA, Christensen RD, Rothstein G (1982) Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol 78:206–209
22. Zhang TY, Ding X, Daynes RA (2005) The expression of 11β-hydroxysteroid dehydrogenase type I by lymphocytes provides a novel means for intracrine regulation of glucocorticoid activities. J Immunol 174:879–889
23. Rachmilewitz D, Simon PL, Schwartz LW, Griswold DE, Fondacaro JD, Wasserman MA (1989) Inflammatory mediators of experimental colitis in rats. Gastroenterology 97:326–337

24. Neurath MF, Becker C, Barbulescu K (1998) Role of NF-κB in immune and inflammatory responses in the gut. Gut 43:856–860

25. Reed KL, Fruin AB, Gower AC, Gonzales KD, Stucchi AF, Andry CD, O'Brien M, Becker JM (2005) NF-κB activation precedes increases in mRNA encoding neurokinin-1 receptor, proinflammatory cytokines, and adhesion molecules in dextran sulfate sodium-induced colitis in rats. Dig Dis Sci 50:2366–2378

26. Andrianifahanana M, Moniaux N, Batra SK (2006) Regulation of mucin expression: mechanistic aspects and implications for cancer and inflammatory diseases. Biochim Biophys Acta 1765:189–222

27. van der Velden VH (1998) Glucocorticoids: mechanisms of action and anti-inflammatory potential in asthma. Mediators Inflamm 7:229–237

28. Pelissier MA, Muller C, Hill M, Morfin R (2006) Protection against dextran sodium sulfate-induced colitis by dehydroepiandrosterone and 7α-hydroxy-dehydroepiandrosterone in the rat. Steroids 71:240–248

29. Whorwood CB, Ricketts ML, Stewart PM (1994) Epithelial cell localization of type 2 11β-hydroxysteroid dehydrogenase in rat and human colon. Endocrinology 135:2533–2541

30. Thieringer R, Le Grand CB, Carbin L, Cai T-Q, Wong B, Wright SD, Hermanowski-Vosatka A (2001) 11β-hydroxysteroid dehydrogenase type 1 is induced in human monocytes upon differentiation to macrophages. J Immunol 167:30–35

31. Sun K, Myatt L (2003) Enhancement of glucocorticoid-induced 11β-hydroxysteroid dehydrogenase type 1 expression by proinflammatory cytokines in cultured human amnion fibroblasts. Endocrinology 144:5568–5577

32. Hardy DB, Pereria LE, Yang K (1999) Prostaglandins and leukotriene B4 are potent inhibitors of 11β-hydroxysteroid dehydrogenase type 2 activity in human choriocarcinoma JEG-3 cells. Biol Reprod 61:40–45

33. Patel FA, Clifton VL, Chwalisz K, Challis JR (1999) Steroid regulation of prostaglandin dehydrogenase activity and expression in human term placenta and chorio-decidua in relation to labor. J Clin Endocrinol Metab 84:291–299

34. Peskar BM, Holland A, Peskar BA (1976) Effect of carbenoxolone on prostaglandin synthesis and degradation. J Pharm Pharmacol 28:146–148

35. Darnel AD, Archem TK, Yang K (1999) Regulation of 11β-hydroxysteroid dehydrogenase type 2 by steroid hormones and epidermal growth factor in the Ishikawa human endometrial cell line. J Steroid Biochem Mol Biol 70:203–210

36. Goppelt-Struebe M (1997) Molecular mechanisms involved in the regulation of prostaglandin biosynthesis by glucocorticoids. Biochem Pharmacol 53:1389–1395

37. Nakase H, Okazaki K, Tabata Y, Uose S, Ohana M, Uchida K, Nishi T, Debreceni A, Itoh T, Kawanami C, Iwano M, Ikada Y (2001) An oral drug delivery system targeting immune-regulating cells ameliorates mucosal injury in trinitrobenzene sulfonic acid-induced colitis. J Pharmacol Exp Ther 297:1122–1128

38. Smith JW, Castro GA (1978) Relation of peroxidase activity in gut mucosa to inflammation. Am J Physiol 234:R72-R79

39. Palmen MJHJ, Dieleman LA, Soesatyo M, Pena AS, Meuwissen SGM, van Rees EP (1998) Effects of local budesonide treatment on the cell-mediated immune response in acute and relapsing colitis in rats. Dig Dis Sci 43:2518–2525

40. Hoebler C, Gaudier E, De Coppet P, Rival M, Cherbut C (2006) MUC genes are differently expressed during onset and maintenance of inflammation in dextran sodium sulfate-treated mice. Dig Dis Sci 51:381–389

41. Jarry A, Vallette G, Branka JE, Laboisse C (1996) Direct secretory effect of interleukin-1 via type I receptors in human colonic mucous epithelial cells (HT29-C1.16E). Gut 38:240–242

42. Trompette A, Blanchard C, Zoghbi S, Bara J, Claustre J, Jourdan G, Chayvialle JA, Plaisance P (2004) The DHE cell line as a model for studying rat gastro-intestinal mucin expression: effects of dexamethasone. Eur J Cell Biol 83:347–358

43. Yoshimitsu M, Hayamizu K, Egi H, Okiyama J, Okajima M, Itamoto T, Asahara T (2006) The neutrophil/Th1 lymphocyte balance and the therapeutic effect of granulocyte colony-stimulating factor in TNBS-induced colitis of rat strains. J Interferon Cytokine Res 26:291–300

44. Togawa J-I, Nagase H, Tanaka K, Inamori M, Umezawa T, Nakajima A, Naito M, Sato S, Saito T, Sekihara H (2002) Lactoferrin reduces colitis in rats via modulation of the immune system and correction of cytokine inbalance. Am J Physiol 283:G187-G195