Local metabolism of glucocorticoids and its role in rat adjuvant arthritis

Peter Ergang[a], Pavel Leden[a,b], Karla Vagnerová[a], Petra Klusoňová[a],
Ivan Mikšík[a], Jana Jurčovičová[c,d], Milan Kment[b], Jiří Pácha[a,*]

[a] Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4 - Krč, Czech Republic
[b] Second Department of Internal Medicine, Third Faculty of Medicine, Charles University, Prague, Czech Republic
[c] Department of Normal, Pathological, and Clinical Physiology, Third Faculty of Medicine, Charles University, Prague, Czech Republic
[d] Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia

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ABSTRACT

11β-Hydroxysteroid dehydrogenase 1 (11HSD1) regulates local glucocorticoid activity and plays an important role in various diseases. Here, we studied whether arthritis modulates 11HSD1, what is the role of pro-inflammatory cytokines in this process and whether altered local metabolism of glucocorticoids may contribute to the feedback regulation of inflammation. Adjuvant arthritis increased synovial 11HSD1 mRNA and 11-reductase activity but treatments with tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) antagonists etanercept and anakinra reduced 11HSD1 upregulation. Treatment with carbenoxolone, an 11HSD inhibitor, increased expression of TNF-α, cyclooxygenase 2, and osteopontin mRNA without any changes in the plasma levels of corticosterone. Similar changes were observed when arthritic rats were treated with RU486, an antagonist of GR. This study suggests that arthritis upregulates synovial 11HSD1, this upregulation is controlled by TNF-α and IL-1β and that the increased supply of local corticosterone might contribute to feedback regulation of inflammation.

1. Introduction

Glucocorticoids are steroid hormones that are known to modulate various immune and inflammatory functions. Both natural and synthetic glucocorticoids have been shown to possess clinical efficacy as anti-inflammatory agents and many immune-mediated diseases are commonly treated with these steroids. There is also a well-established link between glucocorticoids and rheumatoid arthritis (RA). Glucocorticoids are able to suppress synovial inflammation and bone destruction (Kirwan, 1995; Makrygiannakis et al., 2006). Intracellular glucocorticoid concentration in target tissues depends not only on the extracellular concentration, but also on the local metabolism of glucocorticoids, which determines the intracellular concentration of steroids. The bioavailability of biologically active glucocorticoids is controlled by the intracellular enzyme 11β-hydroxysteroid dehydrogenase (11HSD), which has two types – 11HSD1 and 11HSD2. Type 11HSD2 acts exclusively as a dehydrogenase that converts the biologically active glucocorticoids, cortisol and corticosterone, into their inactive 11-oxo derivatives, cortisone and 11-dehydrocorticosterone, respectively, whereas 11HSD1 predominantly mediates the reduction of the 11-oxo derivatives (Draper and Stewart, 2005).

Both types of 11HSDs have been found in bone and synovial tissue; however, 11HSD2 levels were low or barely detected (Cooper et al., 2000; Schmidt et al., 2005). 11HSD1 was demonstrated in human fibroblast-like synoviocytes whereas 11HSD2 expression occurred in macrophage-like synoviocytes (Hardy et al., 2008) and showed higher immunoreactivity in RA patients as compared with healthy controls (Haas et al., 2006).

It has been suggested that 11HSD expression and activity are controlled during inflammation by local feedback regulation. This hypothesis is based on the finding that pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) upregulate 11HSD1 and downregulate 11HSD2 in various cultured cell types (Chapman et al., 2009), including fibroblast-like synoviocytes (Hardy et al., 2006). However, the data have several limitations and these studies prompt more functional analyses to prove whether 11HSDs are involved in feedback regulation of inflammation. First, synovial tissue was obtained from patients who had established RA and were treated with various drugs and no comparison with healthy tissue was done. Second, the effects of pro-inflammatory cytokines on 11HSD1 were shown in synovial cell culture, but the relevance of this effect in intact synovium *in vivo* has not been established. Third, it is unclear whether local activation of biologically active glucocorticoids may contribute to feedback control of synovial inflammation. We therefore examined glucocorticoid metabolism in a rat model of RA and evaluated the effect of corticosterone on synovial inflammation. In particular, we

* Corresponding author. Tel.: +420 24106 2440; fax: +420 24106 2488.
  E-mail address: pacha@biomed.cas.cz (J. Pácha).

addressed the specific potential of TNF-α and IL-1β blockade to interfere with changes in glucocorticoid metabolism during inflammation and the effect of pharmacological inhibition of 11HSD on the expression of inflammatory markers. Based on the large number of cells that travel through the joint during inflammation, glucocorticoid metabolism was also studied in the draining inguinal, lumbar, and caudal lymph nodes.

2. Materials and methods

2.1. Animals, treatments, and evaluation of arthritic damage

Male Lewis rats (7–9 weeks of age, Institute of Physiology, Prague) were kept under a 12/12 h light/dark cycle with unlimited access to chow and tap water. Adjuvant-induced arthritis (Calvino et al., 1999) was induced by a single subcutaneous injection, 2 cm from the base of the tail, of a 100 µl (5 mg/ml) suspension of heat-killed *Mycobacterium butyricum* (Difco Lab., Detroit, MI, USA) in incomplete Freund's adjuvant (paraffin oil:saline:Tween 80 = 1:0.67:0.17).

Two independent experiments were performed. In the first experiment, two groups of animals were compared, rats with adjuvant arthritis and control healthy animals. In the second experiment, the arthritic rats were divided in 5 groups beginning on day 8 after adjuvant injection and treated according to the following protocols for the next 7 days. Group 1 received etanercept, a recombinant TNF receptor-Fc fusion protein (Enbrel, Wyeth, USA); Group 2 received IL-1 receptor antagonist anakinra (Kineret, Amgen, USA); Group 3 received 11HSD inhibitor carbenoxolone (Sigma, USA); Group 4 was treated with glucocorticoid receptor antagonist RU486 (Mifepristone, Sigma) and the last group represented control rats that were treated only with drug vehicle (0.9% NaCl). Carbenoxolone and RU486 were dissolved in ethanol, mixed with 0.9% NaCl and then administered by i.p. (RU486: 30 mg/kg) or s.c. injection (carbenoxolone: 12.5 mg/kg). Etanercept, 4 mg/kg, was also administered by s.c. injection in 0.9% NaCl. Anakinra was delivered by implanted osmotic minipump (model 2ML1, Alzet, Palo Alto, CA, USA) containing a sterile solution of 140 mM NaCl, 10 mM sodium citrate, 0.5 mM EDTA and 0.1% (w/v) Tween 80; the infusion rate was 5 mg anakinra/kg BW/h. The doses were chosen based on recent relevant reports (Coxon et al., 2002; McMullen and Langley-Evans, 2005; Boettger et al., 2008; Watanabe et al., 2008).

The rats were euthanized by cervical dislocation 18 (Experiment 1) or 16 days (Experiment 2) after the induction of arthritis. Blood was withdrawn by heart puncture; draining lymph nodes (inguinal, lumbar, and caudal) and synovial tissue from the knee joints were harvested and frozen in liquid nitrogen or used immediately for isolation of synoviocytes and lymph node immune mobile and stromal cells. Induction and treatment of arthritis was evaluated according to Earp et al. (2008). Shortly, rat's hind paw edema was measured as two cross-sectional areas, one at the base of the metatarsus and the other at the ankle, using a caliper. Two perpendicular measurements were made side-to-side and front-to-back on each section and the ellipse area was calculated. Paw and ankle ellipses were added and normalized to their respective values at day 0. The plasma concentration of corticosterone was measured using a radioimmunoassay kit (MP Biomedicals, Irvine, CA; Beckman Coulter). The experiments were conducted in accordance with the Principles of Laboratory Animal Care and were approved by the Institutional Animal Care and Use Committee.

2.2. RNA extraction, reverse transcription, and quantitative real-time PCR

Total RNA was extracted from synovial tissue and lymph nodes using column isolation with GeneElute Mammalian Total RNA Miniprep Kit (Sigma). Total RNA concentrations were determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The first-strand cDNA was synthesized from RNA using M-MLV reverse transcriptase reagents (Invitrogen, Lofer Austria) and primed with random hexamers (Invitrogen).

The genes analyzed in this study were 11HSD1, 11HSD2, TNF-α, IL-1β, cyclooxygenase 2 (COX-2), osteopontin (OPN), glucocorticoid receptor (GR), and S100A4, a protein associated with RA progression. The levels of specific mRNA were measured using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, USA). The probes and primers used for these experiments were developed as TaqMan Gene Expression Assays by Applied Biosystems, specifically: 11HSD1 (Rn 00567167-m1), 11HSD2 (Rn 00492539-m1), TNF-α (Rn 99999017-m1), COX-2 (Rn 00568225-m1), OPN (Rn 01449972-m1), GR (Rn 00561369-m1), S100A4 (Rn 01451938-m1), and glyceraldehyde-3-phosphate dehydrogenase, GAPDH (TaqMan Endogenous Control Cat. No. 4352338E). The reactions were performed in 30 µl aliquots on a 96-well optical reaction plate containing TaqMan Gene Expression Master Mix with AmpErase UNG (Applied Biosystems), TaqMan probes and cDNA as mentioned earlier (Klusoňová et al., 2009; Ergang et al., 2008). Target gene probes labeled with FAM/TAMRA were used in conjunction with GAPDH probe (VIC/MGB) as a normalization factor. Gene-specific calibration curves were generated from serial dilutions of standard cDNA and mRNA levels of all genes of interest were normalized to the reference gene (GAPDH).

2.3. Isolation of synoviocytes and lymph node cells, and measurement of 11HSD activity

The synovial cells were isolated by enzymatic digestion of fresh synovial tissue according to Firestein and Zvaifler (1987) with minor modifications. The synovial membranes were isolated from the knees under sterile conditions. The tissue was minced and digested in serum-free RPMI-1640 (Biochrom AG, Berlin, Germany) containing 1 mg/ml collagenase type IV (Biochrom AG, Berlin, Germany) for 1 h in a humidified incubator containing 5% CO_2 at 37 °C. The mixture was filtered through a 20 µm nylon mesh, washed extensively by centrifugation and resuspended in RPMI-1640 supplemented with 10% FBS (Biochrom AG, Berlin, Germany) and 0.1% antibiotics (streptomycin, gentamycin, and penicillin) and the freshly digested cells were placed into 6-well plates. Immune mobile and stromal cells were isolated from lymph nodes carefully dissected out of the surrounding fatty acids. Freshly removed nodes were minced with scissors and immune mobile cells were prepared by mechanical disruption – pressing the pieces of tissue against bottom of the Petri dish with the plunger of a syringe until mostly fibrous stromal tissue remains (Kruisbeek, 2000). The disintegrated tissue was filtered through a 20 µm nylon mesh, washed by centrifugation, resuspended in RPMI supplemented with 10% FBS and plated in 8-well plates. The stromal elements which remained on the mesh were collected and treated with collagenase using the protocol identical to the isolation of synoviocytes. Lymph node cells were prepared from pooled samples of inguinal, lumbar, and caudal nodes.

11-Reductase and 11β-oxidase activities were detected over a 24-h time period in RPMI medium containing 21 nmol/l [^{3}H]11-dehydrocorticosterone or [^{3}H]corticosterone to measure 11-reductase or 11β-oxidase activity, respectively. The steroids were extracted from the growth medium using Strata-X C_{18} cartridges (Phenomenex, Torrance, CA, USA) and quantified by HPLC as previously described (Pácha et al., 2004). The protein concentration was determined using the Bradford method.

2.4. Statistical analysis

Results are expressed as means ± SEM and were analyzed by an unpaired Student's *t*-test or by analysis of variance (ANOVA) followed by the Fisher's least significant difference (LSD) test using Statistica 6.1. (StatSoft, Inc., Tulsa, OK, USA). In all cases, a probability level of $P < 0.05$ was considered significant.

3. Results

3.1. The effects of adjuvant arthritis on 11HSD1 in synovial tissue and in draining lymph nodes

The relative paw size rose during progression of arthritis much more than due to natural animal growth and the swelling was observed already on day 10 when the percentage changes in paw normalized to day 0 was 145 ± 10%, whereas the relative paw size of control animals was only 111 ± 5%. The severity of edema increased during the following time course and at day 18 paw swelling reached the value 211 ± 16% (control non-arthritic animals: 125 ± 7%). To investigate whether the acute phase of adjuvant arthritis is associated with changes in glucocorticoid availability in joints we measured expression of 11HSD1 and 11HSD2 mRNA and glucocorticoid metabolism in synovial tissue. As shown in Fig. 1A, expression of 11HSD1 mRNA was more than 20 times higher in the synovium of arthritic rats, compared to control animals. However, 11HSD2 mRNA was not detected in either control or arthritic synovium.

Compared with control animals, the synoviocytes isolated from arthritic rats had greater 11-reductase activity, i.e., the capacity of these cells to activate corticosterone was increased (Fig. 1B). As many cells travel through the joint during inflammation, we also studied the effects of arthritis on 11HSDs in draining lymph nodes. In contrast to the synovium, the nodes of healthy animals expressed both types of 11HSD. Furthermore, adjuvant arthritis significantly stimulated 11HSD1 mRNA (Fig. 2A). 11-Reductase activity was localized to both immune mobile and stromal cells of the lymph nodes (Fig. 2B and C) but arthritis significantly upregulated this activity only in immune mobile cells. In contrast, 11β-oxidase activity was detected predominantly in cells isolated from stromal elements. In keeping with 11HSD2 mRNA expression there was
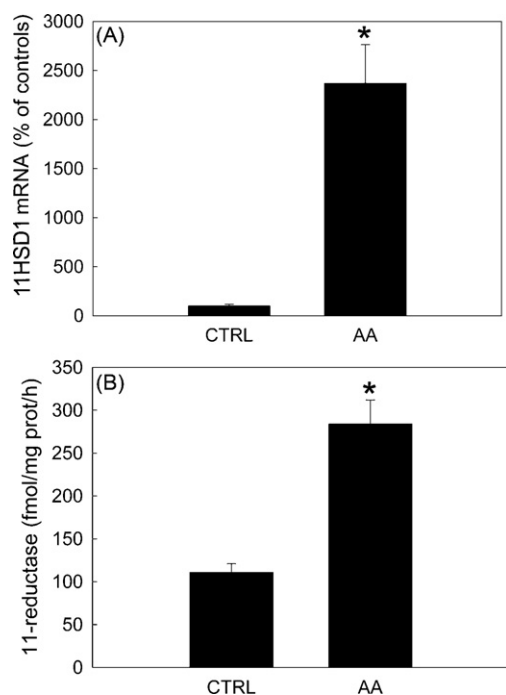
Fig. 1. The effects of arthritis on expression of 11HSD1 mRNA in synovial tissue (A) and 11-reductase activity in primary culture of synoviocytes (B). Mean Ct values in control animals reached 38.4 for 11HSD1 and 32.8 for GAPDH. CTRL, control healthy rats; AA, animals with adjuvant arthritis. Results are means ± SEM (A: $n = 9$, B: $n = 15$). Statistically significant difference: $^*P < 0.01$.

a trend toward decreased inactivation of corticosterone in lymph node stroma of arthritic rats (Fig. 2C).

In order to characterize the severity of local inflammation in synovium and the reaction of lymph nodes, we analyzed the changes in mRNA levels of inflammatory markers TNF-α, IL-1β, COX-2, and OPN. These markers are upregulated in humans and experimental animals with arthritis (Wang and Denhardt, 2008; Brennan and McInnes, 2008). In addition, we quantified the expression of S100A4 calcium-binding protein, which has been identified as a putative factor linking inflammation and pathologic behavior of synovial fibroblasts in arthritis (Ošlejšková et al., 2008). Analysis of the markers by RT-PCR (Fig. 3) showed that arthritis resulted in enhanced expression of all markers studied, in both synovium and lymph nodes. We observed the greatest degree of mRNA upregulation in synovium for TNF-α (∼30×) and S100A4 (∼10×). The mRNAs that were most upregulated in lymph nodes coded for COX-2 (∼30×) and TNF-α (∼5×). In contrast to these genes, the GR mRNA expression was markedly reduced in both synovium (17 ± 4%, $P < 0.01$) and lymph nodes (10 ± 4%, $P < 0.01$) of arthritic rats.

Based on the ability of pro-inflammatory cytokines to stimulate 11HSD1 in cell culture experiments, we questioned whether the *in vivo* upregulation of 11HSD1 reflects the effect of TNF-α and IL-1β. Seven-day treatment of arthritic rats with anakinra, an IL-1 receptor anatagonist, or etanercept, a TNF-α antagonist, significantly decreased 11HSD1 transcript levels (Fig. 4) and edema (Fig. 5).

3.2. Effect of carbenoxolone and RU486 on 11HSD1 expression in synovium and lymph nodes of arthritic rats

To identify whether changes in local metabolism of glucocorticoids may contribute to feedback regulation of inflammation *in vivo*, we tested the effect of carbenoxolone on inflammatory markers. Carbenoxolone is a well-characterized competitive inhibitor of
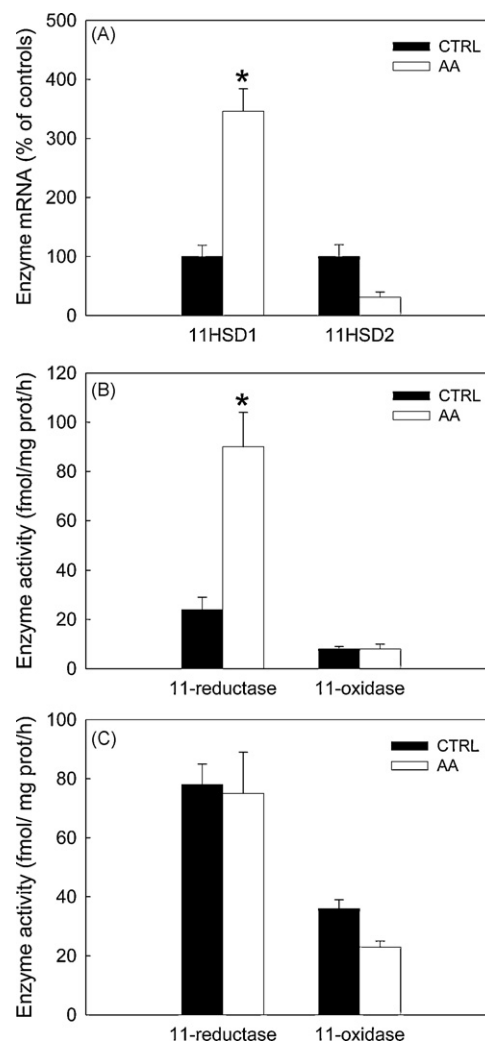

Fig. 2. 11HSD1 and 11HSD2 mRNA expression and 11-reductase and 11β-oxidase activities in lymph nodes of control and arthritic rats. (A) Expression of 11HSD1 mRNA and 11HSD2 mRNA in the lymph nodes; mean Ct values in control animals reached 29.8 for 11HSD1, 35.9 for 11HSD2, and 25.44 for GAPDH. (B) 11-Reductase and 11β-oxidase activity in the immune mobile cells of lymph nodes. (C) 11-Reductase and 11β-oxidase activity in the stromal cells of lymph nodes. CTRL, control healthy rats; AA, animals with adjuvant arthritis. Results are means ± SEM (A: $n = 9$, B: $n = 7$, and C: $n = 5$). Statistically significant difference: $^*P < 0.01$.

11HSD that has been used extensively to study the physiological role of this enzyme *in vivo* and *in vitro* (Young et al., 2003; Liu et al., 2008). Administration of carbenoxolone resulted in exacerbation of edema (Fig. 5) without any changes in the plasma level of corticosterone. Plasma corticosterone level in carbenoxolone (CBX) treated rats was 338 ± 41 ng/ml and in vehicle treated control (CTRL) and arthritic rats (AA) 190 ± 29 and 307 ± 38 ng/ml, respectively (CBX vs. AA, n.s.; CTRL vs. AA, $P < 0.05$). In contrast, carbenoxolone significantly increased expression of inflammatory markers (Fig. 6A). RT-PCR analyses showed a >10-fold increase in TNF-α and COX-2 and a >15-fold increase in OPN in synovium of arthritic rats treated with carbenoxolone as compared to untreated arthritic animals; S100A4 mRNA levels did not change. Similar to carbenoxolone, RU486 increased edema (Fig. 5) and expression of COX-2, and OPN (Fig. 6A); upregulation of TNF-α did not reach significance. These inconsistent effects of carbenoxolone and RU 486 may reflect pharmacokinetic differences in access to synovium.

The increase of synovial transcripts after carbenoxolone and RU486 treatment was not paralleled in lymph nodes. Neither carbenoxolone nor RU486 had any effect on lymph nodes in arthritic
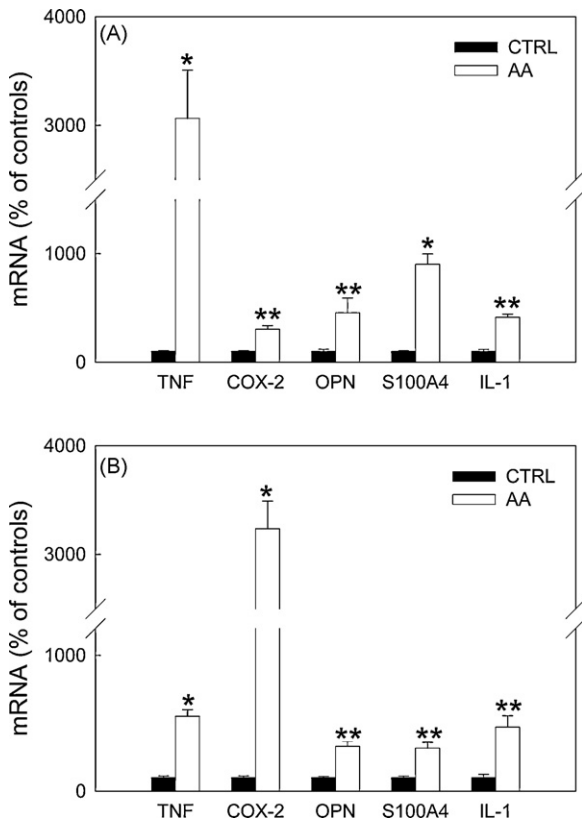
Fig. 3. Expression of tumor necrosis factor α (TNF), cyclooxygenase 2 (COX-2), osteopontin (OPN), protein S100A4, and interleukin 1β (IL-1) in (A) the synovium and (B) the lymph nodes of control and arthritic rats. CTRL, control healthy rats; AA, animals with adjuvant arthritis. Results are means ± SEM ($n = 5$–8). Statistically significant difference: $^*P < 0.01$, $^{**}P < 0.05$.
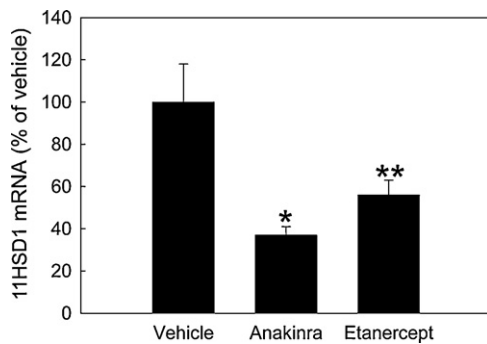

Fig. 4. The effects of IL-1β antagonist anakinra and TNF-α antagonist etanercept on the expression of 11HSD1 mRNA in the synovium of arthritic rats. Results are means ± SEM ($n = 7$). Statistically significant difference: $^*P < 0.01$, $^{**}P < 0.05$.

rats as indicated by not only the level of S100A4 mRNA but also TNF-α mRNA and COX-2 mRNA; only the levels of OPN were increased in carbenoxolone-treated rats (Fig. 6).

4. Discussion

The current study demonstrated the presence of 11HSD1 but not 11HSD2 in healthy rat synovial tissue and the upregulation of 11HSD1 during arthritis. As the changes in expression of 11HSD1 mRNA were accompanied by increased 11-reductase activity, our data indicated that 11HSD1 can provide in vivo synovium with generation of substantial amounts of corticosterone that could damp inflammation. The upregulation of 11HSD1 during arthritis is similar to other in vivo inflammatory responses such as human and
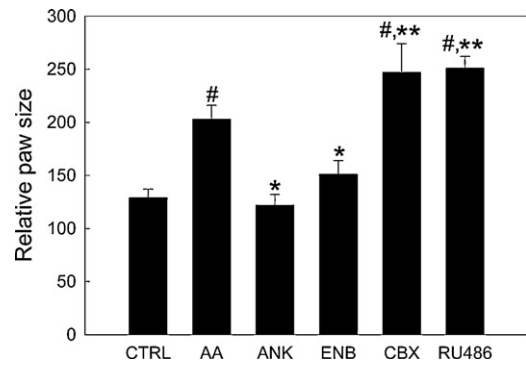

Fig. 5. The effect of anakinra (ANK), etanercept (ETN), carbenoxolone (CBX) and RU486 on relative paw size in arthritic animals. CTRL, control healthy rats; AA, arthritic rats. The vehicle or drugs were given for 7 consecutive days beginning day 8 after induction of arthritis. Bars represent mean values (±SEM, $n = 12$–16) at day 16 relative to their respective start value at day 0. Statistically significant difference: $^\#P < 0.01$ compared to controls; $^*P < 0.01$ and $^{**}P < 0.05$ compared to arthritic rats.

rodent colitis (Žbánková et al., 2007; Vagnerová et al., 2006) and mouse peritonitis (Gilmour et al., 2006). However, the absence of 11HSD2 in rat synovial tissue is inconsistent with previous reports in humans that express both types of 11HSDs; 11HSD1 primarily in fibroblast-like synoviocytes and 11HSD2 in macrophage-like synoviocytes (Schmidt et al., 2005; Hardy et al., 2008). This discrepancy may indicate species or strain differences. In this study we used Lewis rats that are due to blunt HPA axis more susceptible to acute and chronic inflammatory stress than other strains that are much less or quite resistant to development of inflammatory diseases (Sternberg et al., 1989). The lack of glucocorticoid suppression of immunity via HPA has been suggested as an important mechanism that contributes to susceptibility to inflammatory dis-
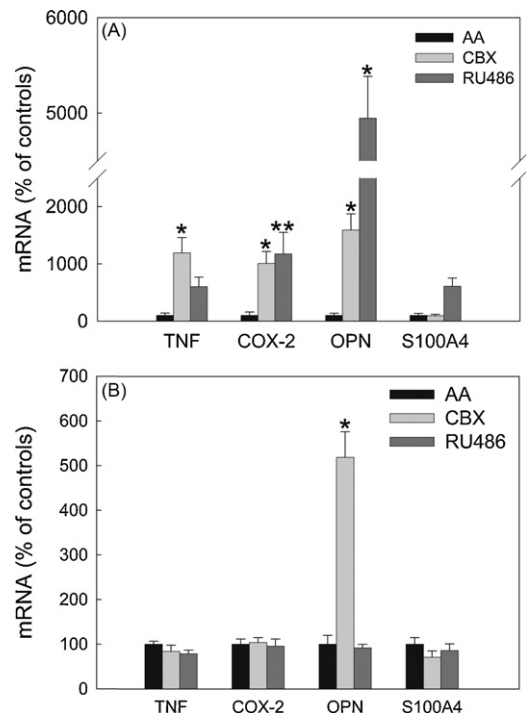

Fig. 6. The effects of treatment arthritic rats with the 11HSD inhibitor carbenoxolone (CBX) and the glucocorticoid receptor antagonist RU486 on mRNA expression of tumor necrosis factor α (TNF-α), cyclooxygenase 2 (COX-2), osteopontin (OPN), and protein S100A4 in (A) the synovium and (B) the lymph nodes. Results are means ± SEM ($n = 5$–6). $^*P < 0.01$ and $^{**}P < 0.05$ compared to control arthritic rats (AA).

eases (Webster et al., 2002) and thus findings in Lewis rats cannot be always extended to other animals. Nevertheless, it seems that there are no major strain differences in the sensitivity of target tissues to glucocorticoids even if HPA responsiveness differs markedly (Karalis et al., 1995).

During the course of inflammation, activated macrophages produce TNF-α, IL-1, and other cytokines and stimulate the recruitment of blood cells and the proliferation of synovial cells to form a pannus that degrades bone via the activation of osteoclasts. Similarly, the synovium of rats with adjuvant arthritis showed increased TNF-α mRNA and IL-1β mRNA levels (Fig. 3A). Numerous *in vitro* studies have shown that these cytokines are potent inducers of 11HSD1 in various cell culture systems, including cultures of isolated synoviocytes (Escher et al., 1997; Tomlinson et al., 2001; Cooper et al., 2001; Hardy et al., 2006). To support the hypothesis that TNF-α and IL-1β play a role in upregulation of 11HSD1 following joint inflammation, we treated the arthritic rats with anakinra, an IL-1 receptor antagonist, and etanercept, a TNF antagonist. We found that these drugs not only improved the clinical presentation of animals but also decreased upregulation of 11HSD1. In rats with adjuvant arthritis, both anakinra and etanercept have previously been found to decrease pathological angiogenesis and paw swelling, to alter histological parameters, and to modulate thermal and mechanical hyperalgesia (Coxon et al., 2002; Zwerina et al., 2004; Inglis et al., 2005; Boettger et al., 2008). Thus, it appears that partial inhibition of paw edema and 11HSD1 is achievable by blocking both TNF-α and IL-1β. There are several mechanisms by which anti-cytokine therapy is known to act in human RA. We can hypothesize that anti-cytokine treatment modulates the local glucocorticoid metabolism not only by interaction with resident synovial cells but also by preventing migration of cells into the joint. Importantly, however, the cellular composition of inflamed tissue during experimental arthritis was not modulated by anti-TNF or anti-IL-1 treatment even if the effector molecules of cytokine-driven synovial inflammation decreased significantly after blockade of cytokines (Zwerina et al., 2004).

The activity of glucocorticoids is mediated via GR expression, which is significantly decreased in various inflammatory processes (Kamiyama et al., 2008). This decrease can be observed also in the synovium of collagen- (Miller et al., 1995) and adjuvant-induced arthritis (this study). We therefore sought to determine whether the upregulation of 11HSD1 has any physiological significance during inflammation. As treatment with glycyrrhetinic acid or its soluble derivative carbenoxolone is known to decrease both 11-reductase activity (Hu et al., 2008) and 11HSD1 mRNA expression *in vivo* (Whorwood et al., 1993; Liu et al., 2008), we examined the possibility that the expression of inflammatory markers was upregulated in carbenoxolone-treated arthritic rats. In our hands, this treatment resulted in a significant exacerbation of arthritis and increase of synovial TNF-α mRNA, COX-2 mRNA, and OPN mRNA, with no change in plasma levels of corticosterone. It is well known, that TNF-α and COX-2 are downregulated by biologically active glucocorticoids (Webster et al., 2002) and OPN is upregulated by various factors including TNF-α and IL-1β (Wang and Denhardt, 2008).

Carbenoxolone is a nonspecific inhibitor and it is possible that the effect of the drug on TNF-α, COX-2, and OPN expression may be attributable to proteins other than 11HSD such as gap junctions. Although carbenoxolone is a potent gap junction blocker, it is outside that the effect of carbenoxolone was associated with gap junctions. The action on connexins requires drug concentration higher than micromolar, usually 10^{-5} M (Davidson and Baumgarten, 1988; Matemba et al., 2006) but the concentration of carbenoxolone in interstitial fluid after drug doses similar to ours reached only nanomolar range (Tomlinson et al., 2007). Thus the interstitial concentration of carbenoxolone in our experiments

was not able to interfere with connexins but was high enough to be in excess to physiological concentration of free corticosterone and thus to be engaged in competitive inhibition of 11HSD1. It is very likely therefore that carbenoloxone inhibited 11HSD1 similar as in human studies (Sandeep et al., 2005; Tomlinson et al., 2007). Further, carbenoxolone did not alter plasma corticosterone. Against this background, exacerbation of arthritis with carbenoxolone cannot readily be attributed to effects outside the synovium or to large changes in circulating corticosterone level. It is likely therefore that synovial cells use less corticosterone in the presence of carbenoxolone even if nonspecificity of the drug cannot exclude other mechanisms.

In contrast to synovial tissue, carbenoxolone treatment in lymph nodes did not upregulate the expression of TNF-α or COX-2, although 11HSD1 was found in lymphoid organs including lymph nodes (Hennebold et al., 1996; Vagnerová et al., 2006). Our observation of 11HSD1 upregulation in lymph nodes of arthritic rats and the lack of any carbenoxolone and RU486 effect suggest the possibility that at least some cells within the lymph nodes are resistant to glucocorticoids or that there are pharmacodynamic differences in access to lymph nodes and synovium. Detailed assessment of the functional implications of 11HSD1 expression is beyond the scope of this study, but we cannot exclude the possibility of significantly decreased cellular sensitization to glucocorticoids. In contrast to TNF-α mRNA and COX-2 mRNA expression, the level of OPN mRNA was significantly enhanced in carbenoxolone-treated rats. At present we do not have any explanation for the discrepancy between the effect of carbenoxolone on the expression of OPN, TNF-α and COX-2 in arthritic rats. There may be regional differences between regulation of OPN in synovium and lymph nodes because regulation of OPN expression is complex and tissue specific (Wang and Denhardt, 2008). Our interpretation of these findings, taken together, is that although 11HSD1 is upregulated in both the synovium and the draining lymph nodes, this enzyme might be potent enough to restrain some inflammation-associated processes in the synovium but not in the peripheral lymphoid organs.

In conclusion, the current study demonstrates *in vivo* control of synovial 11HSD1 by pro-inflammatory cytokines TNF-α and IL-1β during arthritis and indicates a differential role of 11HSD1 upregulation in target tissue and in peripheral lymphoid organs during inflammation.

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