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Enhanced ecto-apyrase activity of stimulated endothelial or mesangial cells is downregulated by glucocorticoids in vitro

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Abstract

Endothelial as well as mesangial cells show enhanced activity of ecto-apyrase following pro-inflammatory stimulation in vitro. Since this ecto-enzyme appears to be able to regulate plasma hemopexin, which latter molecule plays a role in the pathogenesis of corticosteroid responsive nephrotic syndrome, the question was raised whether glucocorticoids are potentially able to downregulate ecto-apyrase activity of these cells. Therefore, cell cultures of endothelial or mesangial were stimulated with or without lipopolysaccharide (10 ng/ml). Parallel cultures were supplemented with prednisolone with or without the glucocorticoid receptor antagonist mifepristone in various concentrations. After 24 h, cytospins were prepared and cytochemically stained for ecto-apyrase activity. mRNA for apyrase of these cells was detected using reverse transcription–polymerase chain reaction (RT-PCR). Apyrase activity of either cells or soluble apyrase (0.16 U/ml buffer) with or without supplementation of prednisolone were biochemically assayed for their phosphatase activity. The results show significantly decreased ecto-apyrase activity of lipopolysaccharide-stimulated cells after treatment with prednisolone as compared to non-prednisolone-treated cells. Preincubation with mifepristone did not inhibit the effect of prednisolone. Identical mRNA signals for apyrase were found in prednisolone and non-prednisolone-treated cells. Interestingly, soluble apyrase also showed a significant decrease of activity following preincubation with prednisolone. It is concluded that prednisolone is able to downregulate ecto-apyrase of stimulated endothelial or mesangial cells, which may potentially inhibit the conversion of hemopexin to its pro-inflammatory isoform. As blocking of the cytosolic glucocorticoid receptor showed no effect upon the prednisolone action, whereas prednisolone is able to affect soluble apyrase per se, it is felt that this particular action of prednisolone may (at least partly) be mediated through a non-genomic pathway.
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1. Introduction

Ecto-apyrase (CD39) belongs to a family of ecto-nucleoside phosphatases and is designated as NTPase-1 according to a recently proposed nomenclature (Zimmermann et al., 2000). It is present along the surface of vascular endothelium and upon various other cell types including the glomerular mesangium (Enjyoji et al., 1999; Sevigny et al., 2000).

Various functions have been attributed to this ATP-diphosphohydrolase which is able to degrade extracellular nucleotides released from either activated platelets (Mills et al., 1968; Gordon, 1986), or from red blood cells (Bodas et al., 2000; Sprague et al., 2001; Abraham et al., 2001; Jagger et al., 2001) or from vascular endothelial cells (Pearson and Gordon, 1979, 1985; Milner et al., 1990; Yang et al., 1994).

Thus, Marcus et al. (1997) have shown anti-thrombotic activity of ecto-apyrase, hydrolysing the pro-aggregatory

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nucleotide ADP, whereas hydrolysis of extracellular ATP by ecto-apyrase preventing the pro-inflammatory stimulation of neutrophils by extracellular ATP through their P2Y receptors has been observed as well (Ward et al., 1988; Bakker et al., 1994; Poelstra et al., 1992).

It has been suggested that ecto-apyrase may also be associated with ATP release besides its role in ATP hydrolysis (Wang et al., 1998; Bodas et al., 2000) together with other proteins of the super family of ATP binding cassette (ABC) membrane proteins (Abraham et al., 2001).

Recently, we observed that an enzymatic isoform of the acute phase protein hemopexin could be inactivated by extracellular nucleotides like ADP or ATP. The protease activity of this inactivated hemopexin could be restored by treatment with soluble apyrase yielding the enzymatic active form of this molecule (Bakker et al., 2000).

It appeared that lipopolysaccharide-stimulated endothelial cells, which show enhanced ecto-apyrase activity (Kittel, 1999), were also able to reactivate inactivated hemopexin to active hemopexin following incubation of these cells with inactivated hemopexin in vitro (Kapojos et al., 2004). In other words, enhanced endothelial ecto-apyrase may promote the activation of this pro-inflammatory isoform of hemopexin. This may be an example of the close interaction between plasma enzymes and plasma membrane associated enzymes of endothelial cells leading to either homeostasis or, in case of derangement, to enhanced vascular permeability.

Interestingly, active hemopexin has been implicated in the pathogenesis of corticosteroid responsive nephrotic syndrome, since enhanced activity of plasma Hx occurs in subjects with corticosteroid responsive nephrotic syndrome in relapse as compared with plasma of subjects in remission (manuscript in preparation). In addition, active hemopexin is also able to induce both corticosteroid responsive nephrotic syndrome like glomerular lesions as well as proteinuria in rats following intra-renal infusion in vivo (Cheung et al., 2000).

The pathogenesis of corticosteroid responsive nephrotic syndrome is unknown; T cell associated immune mechanisms are supposed to be involved; the beneficial effect of treatment with prednisolone or immunosuppresants in most patients with corticosteroid responsive nephrotic syndrome fits well within this concept (Glassock, 2003). It appeared from preliminary results that treatment of stimulated endothelial cells in vitro with prednisolone resulted in downregulation of ecto-apyrase activity. Thus, in addition to corticosteroid mediated effects upon immune cells (Schimmer and Parker, 2001), also modulation of endothelial or mesangial ecto-apyrase may occur by corticosteroids, which may result in an inhibited conversion of inactivated hemopexin to active hemopexin.

To confirm and extend this potential mechanism, we now evaluated the effect of prednisolone upon the expression and activity of endothelial and mesangial ecto-apyrase in vitro. It is shown that prednisolone significantly downregulated the expression and activity of stimulated endothelial and mesangial ecto-apyrase; which effect may partly due to a non-genomic mechanism of corticosteroid action.

2. Materials and methods

2.1. Endothelial cells in vitro

Human umbilical cord venous endothelial cells obtained from Dr. G. Molema (Endothelial Cell Facility Groningen University/Academic Hospital Groningen, The Netherlands) were cultured in RPMI 1640 (BioWhittaker) supplemented with 20% fetal calf serum (Integro) and under standard conditions with minor modifications (Jaffe et al., 1973). Only lower passage numbers (1–3) in either 6-well plates (Costar) or chamber slides (LabTek slides; Nalge Nunc International) were used throughout the experiments.

Confluent cells cultures were stimulated with lipopolysaccharide (10 ng/ml culture medium; BioWhittaker) in the presence or absence of prednisolone disodium phosphate 2.5 mM (Schmid et al., 2000) or with vehicle alone, at 37 °C. [The glucocorticoids used in this study were obtained from the university hospital pharmacy, Groningen, prepared according to Ph. Eur. IV.] In another set of experiments, cultures were preincubated with mifepristone (0.5 μM) for 60 min; Sigma-Aldrich). Mifepristone when used as an antagonist of the cytosolic glucocorticoid receptor (Yu et al., 2002; Shoupe et al., 1987; Gaillard et al., 1984; Spitz and Bardin, 1993). This glucocorticoid receptor is present in human endothelium (Ji et al., 2003; Yu et al., 2002). Also lower doses of prednisolone were tested: lipopolysaccharide-stimulated cell cultures were compared following treatment with either prednisolone alone (2.5 mM or 2.5 μM); or prednisolone in combination with mifepristone (12.5 μM or 12.5 nM, respectively). After 24 h, the monolayers were washed with Hank's balanced salt solution (HBSS; GIBCO BRL), carefully detached using a disposable cell scraper and cytospins were prepared according to standard methods and stained for apyrase activity using a cytochemical method. Only cell suspensions in which the viability was >96%, as tested by dye exclusion, were used. A part of the cells were snap-frozen using isopentane and kept at −80 °C until use for RNA isolation. Chamber slides were prepared and fixed according to standard methods and stained for apyrase expression by immunostaining. In another set of experiments, endothelial cell monolayers were washed and tested biochemically for apyrase activity. In this assay also, lower amounts of prednisolone with or without mefipristone were tested (2.5 μM prednisolone alone, or in combination with mifepristone (2.5 mM prednisolone with 12.5 μM mifepristone as well as 2.5 μM prednisolone with 12.5 nM mifepristone).

2.2. Mesangial cells in vitro

As preliminary studies showed identical responses after stimulation with lipopolysaccharide or cytokines with respect to ecto-apyrase expression and activity in primary human mesangial cells versus immortalized human mesangial cells, we used in the present study this particular cell line (Banas et al., 1999). Cells were grown in Dulbecco's modified Eagle's medium (DMEM; BioWhittaker) supplemented with 10% fetal calf serum and 1% normal human serum in tissue culture flasks according to the protocol of Banas et al. (1999) with minor modifications. The identity of the cells was established by morphological criteria and positive immunostaining for smooth muscle actin, fibronectin and negative immunostaining for factor VIII and vimentin. Confluent cultures were prepared in flask (25 cm^2; Costar), subsequently stimulated with either lipopolysaccharide 10 ng/ml or vehicle alone with or without prednisolone (2.5 mM) for 24 h. Since it is uncertain known whether the mesangium cell-line used possesses the cytosolic glucocorticoid receptor, experiments with mifepristone were confined to endothelial cell cultures.

The monolayers were prepared for cytospins or biochemical assay as mentioned previously under "endothelial cells in vitro".

2.3. Immunostaining of ecto-apyrase expression

Cytospins or chamber slides were fixed in acetone for 10 min at −20 °C, and stained for ecto-apyrase according to standard methods (Cheung et al., 1996b), using monoclonal mouse anti-apyrase antibody as a first step and subsequently peroxidase-conjugated goat-anti-mouse antibody (Pierce) as a second step. Reaction product was visualized by 3-amino-9-ethyl-carbazole (Aldrich Chemical). All incubation steps of the staining procedures were carried out at 20 °C. Cytospins were counterstained with haematoxyline (Merck).

2.4. Cytochemical demonstration of ecto-apyrase activity

Apyrase activity was demonstrated by staining of cytospins using the cerium-based method as described previously (Cheung et al., 1996a). Briefly, cytospins were fixed with pararosaniline (Sigma) and after preincubation with cerium (Fluka Chemie), incubated with reaction mixture containing 2.3 mM ADP (Sigma) at 37 °C. Precipitated cerium phosphate was visualized according to standard methods (Cheung et al., 1996a).

2.5. Biochemical apyrase assay

Cellular apyrase activity was biochemically assayed in stimulated or non-stimulated cell cultures using ADP as a substrate as described elsewhere (Lowry et al., 1957). In brief, culture medium of monolayer of either endothelial cells (4×10^5 cells/well) or mesangial cells (1.3×10^6 cells/flask) was discarded, the monolayers were washed with saline twice and subsequently incubated with 200 μl Tris/HCl buffer (0.015 M, pH 8.2; Merck) with 0.015 M 2-amino-2-methyl-1.3 propandiol (Sigma-Aldrich) and 1 mM $MgCl_2$ (Merck) supplemented with ADP (2.5 mM) at 37 °C. Parallel incubations were performed without substrate. The reaction was stopped after 60 min, by quickly freezing the mixture at −20 °C. Inorganic phosphate was subsequently measured in relation to protein content (Lowry et al., 1951) according to the method of ChandraRajan and Klein (1976).

The activity of soluble apyrase (Sigma) was also tested using the standard assay. Soluble apyrase (0.16 U/ml) was mixed with the same mixture as described above with or without preincubation with prednisolone or dexamethasone disodium phosphate (both 2.5 mM). Incubation with either substrate (ADP; 2.5 mM) alone or ADP with equal amounts of either prednisolone or dexamethasone served as controls.

2.6. Detection of CD39 mRNA in endothelial cells

Total RNA was isolated from frozen endothelial cells, from either non-stimulated or lipopolysaccharide-stimulated cells with or without prednisolone treatment, according to the protocol from the manufacturer (Absolutely RNA™ RT-PCR Miniprep Kit; Stratagene, Amsterdam, The Netherlands). Routinely, a DNAse treatment step was included in the RNA isolation procedure.

The cDNA synthesis was primed with oligo(dT) using the protocol provided by the manufacturer (Invitrogen). CD39 primers were selected from the sequence present in the GenBank (accession number: U87967). CD39 primer sequences used for the amplification were f 5′-CAGTTACTGCCCTTACTCCC-3′ and r 5′-CATGTAGCCCAAAGTCCAGCC-3′. PCR for CD39 and GAPDH was performed with 1 unit of Taq-polymerase (Amersham Biosciences Europe) and the reaction buffer provided by the manufacturer. The PCR program of CD39 consisted of 25 cycles with a denaturation step of 30 seconds at 94 °C, an annealing step of 45 s at 58 °C, and an extension step of 45 s at 72 °C. The first denaturing step lasted for 5 min and the final extension step lasted for 7 min. Amplification of GAPDH was performed for 18 cycles and used as a control for cDNA synthesis and to compare mRNA amounts.

3. Results

3.1. Endothelial apyrase

Fig. 1 shows apyrase activity of lipopolysaccharide-treated endothelial cells with or without incubation with prednisolone. A significant increase of phosphate release can be measured in the supernatants of stimulated cells reflecting apyrase activity, whereas the addition of prednisolone to these cells significantly decreases their activity. Cytochemical studies detecting apyrase activity confirm

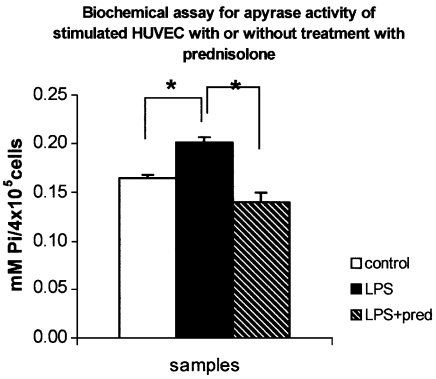

Fig. 1. Cells were incubated for 24 h with either medium (open column), lipopolysaccharide (10 ng/ml, solid column) or lipopolysaccharide (10 ng/ml)+prednisolone (2.5 mM, hatched column). The columns represent arithmetic means of apyrase activity as reflected by the phosphate release measured in each group of cell cultures (n=4). It can be seen that while a significant increase of activity occurs after stimulation with lipopolysaccharide (solid column versus open column), the cells incubated with lipopolysaccharide+prednisolone show significant lower amount of activity as compared with lipopolysaccharide-stimulated cells alone (hatched versus solid column) (*$P \leq 0.05$, Wilcoxon). Bars represent standard deviation.

these findings. In Fig. 2, a similar increase of ecto-apyrase is shown in lipopolysaccharide-stimulated endothelial cells (Fig. 2B) as compared to non-stimulated cells (Fig. 2A), whereas stimulated cells treated with prednisolone showed downregulation of the enhanced ecto-apyrase activity (Fig. 2C). Addition of mifepristone to prednisolone-treated stimulated cells showed similar staining as control cells (Fig. 2D). Immunostaining using anti-apyrase antibodies reflecting changes in protein expression rather than enzyme activity of endothelial cells show an equal staining pattern (Fig. 3). Again upregulation of ecto-apyrase expression occurs following stimulation with lipopolysaccharide (Fig. 3A versus B), whereas prednisolone treatment with or without mifepristone showed the same level of ecto-apyrase expression as seen in control cells (Fig. 3C and D). Mifepristone alone did not affect ecto-apyrase expression or activity of lipopolysaccharide-stimulated or non-stimulated endothelial cells (results not shown). From Fig. 4, it can be seen that also lower doses of prednisolone can induce significant reduction of apyrase activity, whereas preincubation of these cultures with mifepristone did not significantly alter the phosphate release in this experiments.

Analysis of the mRNA signal for ecto-apyrase in lipopolysaccharide-stimulated cells with or without prednisolone using RT-PCR, however revealed no reduced signal for apyrase in stimulated cells treated with prednisolone as compared with lipopolysaccharide-stimulated cells alone (Fig. 5).

3.2. Mesangial apyrase

Lipopolysaccharide-stimulated mesangial cells following prednisolone treatment show also decrease of apyrase activity as compared to lipopolysaccharide-stimulated cells without treatment with prednisolone using the biochemical apyrase assay (Fig. 6). In Fig. 7, activity of ecto-apyrase is depicted using cytochemical staining whereas Fig. 8 shows ecto-apyrase expression detected by immunostaining. It is shown that both reduced activity (Fig. 7) as well as reduced expression of apyrase (Fig. 8) are found after prednisolone

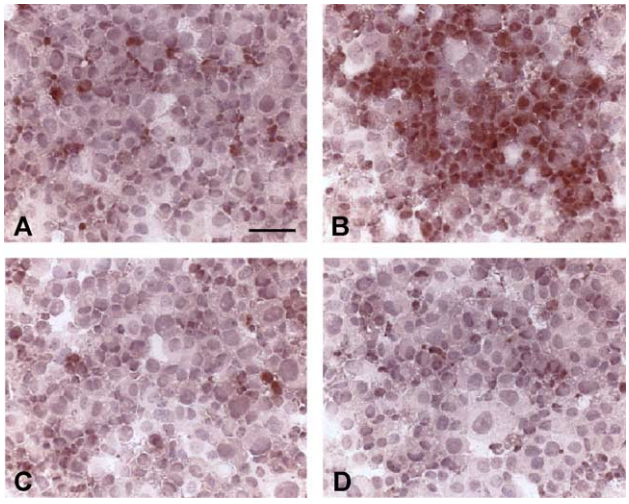

Fig. 2. Cytochemically demonstrated apyrase activity on cytospins of human umbilical cord venous endothelial cell cultures treated with either medium (A), lipopolysaccharide (10 ng/ml, B), lipopolysaccharide (10 ng/ml)+prednisolone (2.5 mM, C), or lipopolysaccharide+prednisolone following mifepristone (0.5 μM) treatment (D); photomicrographs show increased reaction product after lipopolysaccharide stimulation (B) as compared to either non-stimulated cells (A), or stimulated cells treated with either prednisolone (C) or prednisolone+mifepristone (D). Bar=50 μm.

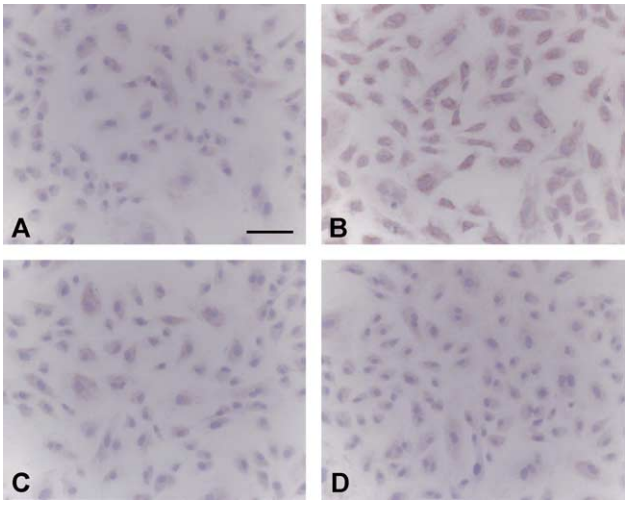

Fig. 3. Apyrase expression of cultured human umbilical cord venous endothelial cells treated with either medium (A), lipopolysaccharide (B), lipopolysaccharide+prednisolone (C), or mifepristone followed by lipopolysaccharide+prednisolone (D), using standard immunostaining with monoclonal anti-apyrase antibody. Photomicrographs show increased reaction product in lipopolysaccharide-stimulated cells (B) as compared to either non-stimulated cells (A) or stimulated cells treated with either prednisolone (C) or prednisolone+mifepristone (D). Bar=100 μm.

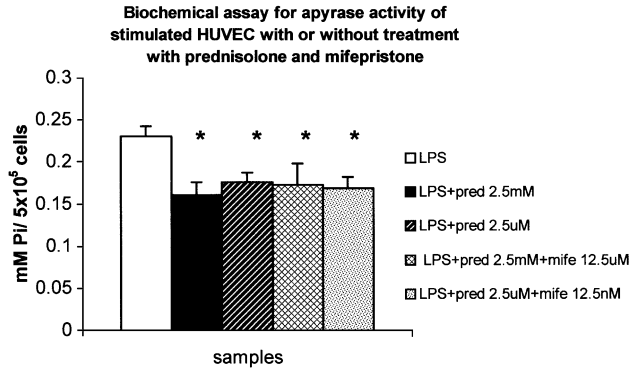

Fig. 4. Cells were incubated for 24 h with either lipopolysaccharide in culture medium (10 ng/ml, open column) or equal amounts of lipopolysaccharide supplemented with either prednisolone, 2.5 mM (solid column), or 2.5 μM (hatched column), or prednisolone+mifepristone (2.5 mM and 12.5 μM, respectively; double hatched column), or prednisolone+mefipristone (2.5 μM and 12.5 nM, respectively; stippled column). The columns represent arithmetic means of apyrase activity as reflected by phosphate release measured in each group of cell cultures (n=5). It can be seen that while a significant decrease of activity occurs in lipopolysaccharide-stimulated cultures supplemented with prednisolone (with or without mifepristone), as compared with the lipopolysaccharide cultures without supplementation of drugs (* versus open column), no significant differences are detectable between cultures with prednisolone alone versus prednisolone in combination with mifepristone (solid column versus hatched, double hatched or stippled columns) (*P<0.01 Wilcoxon; bars represent standard deviation).

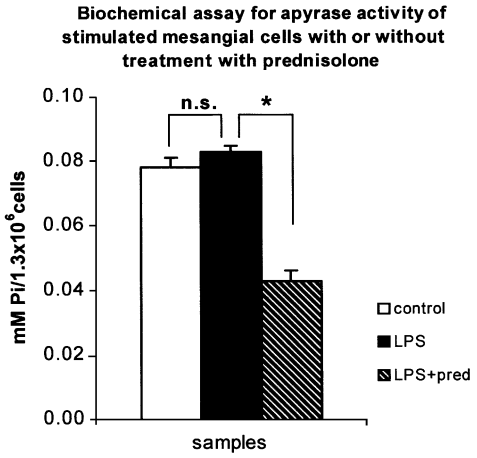

Fig. 6. Mesangial cells were incubated for 24 h with either medium (open column; n=3), lipopolysaccharide (10 ng/ml, solid column; n=5) or lipopolysaccharide (10 ng/ml)+prednisolone (2.5 mM, hatched column; n=5). The columns represent arithmetic means of apyrase activity as reflected by the phosphate release measured in each group of cell cultures. It can be seen that the lipopolysaccharide+prednisolone-incubated cells show significant lower amount of activity as compared with lipopolysaccharide-stimulated cells without treatment with prednisolone (hatched versus solid column) (*P≤0.05, Wilcoxon). The mean increase of lipopolysaccharide-stimulated cells versus non-stimulated cells (solid versus open column) is statistically not significant (n.s.). Bars represent standard deviation.

treatment of lipopolysaccharide-stimulated cells as compared to stimulated cells without prednisolone.

3.3. Soluble apyrase

In Fig. 9, apyrase activity of soluble apyrase solution as detected by a standard biochemical assay is depicted. Phosphate release was measured using ADP as a substrate with or without supplementation of prednisolone versus buffer solution alone or buffer solution with prednisolone. It can be seen that a significant decrease in phosphate release occurs following supplementation of the apyrase with prednisolone, while buffer alone with or without prednisolone shows no significant activity. A similar effect was also seen after addition of dexamethasone (2.5 mM) to the apyrase solution although to a lesser extent (results not shown).

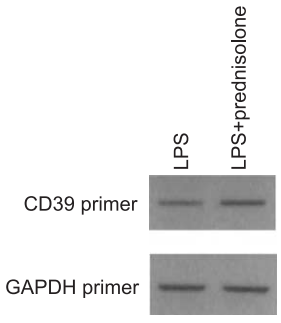

Fig. 5. Gel electrophoresis of apyrase RT-PCR products from lipopolysaccharide-stimulated endothelial cells with or without prednisolone treatment (upper panel). The lower panel shows RT-PCR products of GAPDH (housekeeping gene). No significant differences of apyrase mRNA signals can be seen after prednisolone treatment of lipopolysaccharide-stimulated cells versus lipopolysaccharide-stimulated cells alone (upper panel).

4. Discussion

The aim of the present study was to confirm and extend our preliminary observation suggesting that glucocorticoids may be able to downregulate ecto-apyrase of stimulated endothelial or mesangial cells.

Therefore, we stimulated human umbilical cord venous endothelial cells as well as human mesangium cells with lipopolysaccharide with or without treatment with prednisolone in vitro. It is clear from Figs. 1–3 that the ecto-apyrase expression (Fig. 3) as well as activity (Figs. 1 and 2) of endothelial cells are inhibited following treatment with prednisolone. An identical effect was observed when dexamethasone was used instead of prednisolone (unpublished observations). As can be seen from Figs. 6–8, similar downregulation of ecto-apyrase occurs in mesangial cells following treatment with prednisolone in vitro. The non-significant difference between unstimulated and lipopolysaccharide-stimulated mesangial cells shown in Fig. 6 may be due to the lack of CD14 receptor in these cultures (Yang et al., 1999).

Interestingly, mifepristone which is an antagonist of the cytosolic glucocorticoid receptor, (Yu et al., 2002; Spitz and Bardin, 1993), was not able to block the effect of

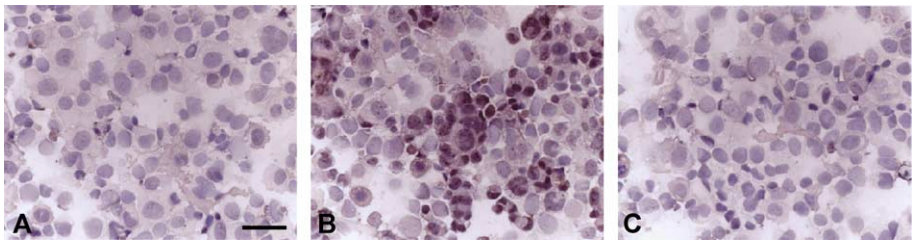

Fig. 7. Photomicrographs of mesangial cell cytospins cytochemically stained for apyrase activity. It can be seen that apyrase activity as reflected by brown reaction product occurs predominantly in lipopolysaccharide-stimulated cells (B) as compared with unstimulated cells (A). Lipopolysaccharide-stimulated cells treated with prednisolone (C) stain also to the same level as compared to non-stimulated control cells (A). Bar=50 μm.

prednisolone (Figs. 2D and 3D). In addition, lower concentrations of prednisolone were able to decrease the ecto-apyrase activity in endothelial cells, whereas mifepristone tested in the prednisolone/mifepristone ratio of 200, has no effect upon the reduced apyrase activity due to prednisolone.

The classical glucocorticoid effect occurs through binding of the steroid to the cytosolic receptor protein leading to an activated steroid-receptor complex which enters the nucleus and binds specific regulatory sequences in the DNA. This binding to the DNA gives rise to gene transcription (Schimmer and Parker, 2001). Since mifepristone did not result in a detectable decrease of the mRNA signal, it is likely that the present action of prednisolone occurs (at least partly) at the protein level rather than at the transcription level. This is in line with the data depicted in Fig. 5, showing no differences in mRNA signals for ecto-apyrase in stimulated endothelial cells with or without treatment with prednisolone. mRNA signals for apyrase of stimulated mesangial cells with or without prednisolone showed identical results (not shown).

If in the present experiments prednisolone acts through a non-genomic pathway, the most likely target is the ATP-driven Ca^{2+} pump, which is identical to ecto-apyrase (Chaudhary et al., 2001; Dhalla and Zhao, 1988).

To explore the possibility that prednisolone affects this enzyme per se, we also tested the effect of prednisolone upon soluble apyrase activity.

It is clear from Fig. 9 that prednisolone caused a highly significant reduction of the soluble apyrase activity as such; this finding supports the concept of a direct effect of corticosteroids upon the membrane associated enzyme in vitro.

Non-genomic corticosteroid effects have been described by other authors as well although the mechanism remains greatly unknown (Croxtall et al., 2000; Buttgereit and Scheffold, 2002). Reduced activity of membrane associated ATPases, including Ca^{2+}-dependent ATPase, due to glucocorticoids may be mediated through altering the physicochemical properties of the cell membrane in such a way that ion flux across the membrane is reduced (Schmid et al., 2000). Also in endothelial cells of coronary arteries in vitro, Rogers et al. (2002) observed a direct influence upon calcium mobilisation caused by these drugs. In other endothelial cell cultures, inhibition of calcium mobilisation due to dexamethasone has been observed (Rosenstock et al., 1997). It is conceivable therefore that the effect of prednisolone upon endothelial or mesangial ecto-apyrase activity in vitro relates to a conformational change of the enzyme molecule mediated via calcium or magnesium ions, which cations are essential for ecto-apyrase activity (Plesner, 1995; Nagy et al., 1997; Chen and Guidotti, 2001).

In rats, upregulation of ecto-apyrase activity has been observed in endothelium of brain capillaries in rats (Kittel, 1999) and in human mesangial cells in vitro (Kapojos et al., 2004) following treatment with lipopolysaccharide or tumor necrosis factor-α (TNF-α).

Up to now, downregulation of enhanced ecto-apyrase expression in stimulated cells by anti-inflammatory drugs has not been described. The classical anti-inflammatory effect of glucocorticoids is mediated through the cytosolic

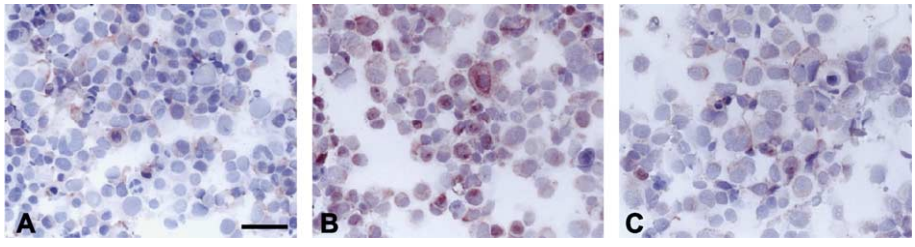

Fig. 8. Apyrase expression of mesangial cells treated with either medium (A), lipopolysaccharide (B), or lipopolysaccharide+prednisolone (C) using standard immunostaining for ecto-apyrase. Increased reaction product is shown in lipopolysaccharide-stimulated cells (B), whereas control cells (A) or cells treated with lipopolysaccharide+prednisolone (C) show less staining. Bar=50 μm.

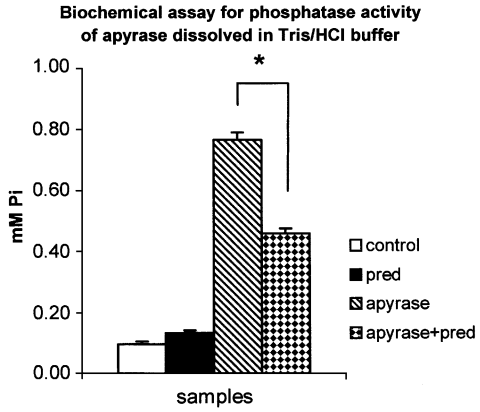

Fig. 9. Apyrase activity was tested using ADP as a substrate; control activity is shown in buffer alone (open column), prednisolone in buffer (2.5 mM, solid column), apyrase (0.16 U/ml, hatched column) or apyrase (0.16 U/ml) mixed with prednisolone (2.5 mM, stippled column). Columns represent arithmetic means of phosphate release in each set of experiments (n=8). It can be seen that while buffer (open column) or prednisolone (solid column) do not show significant phosphatase activity, apyrase solution shows significant phosphate release which is inhibited when apyrase is preincubated with prednisolone (*P<0.01; Wilcoxon). Bars represent standard deviation.

glucocorticoid receptor which occurs in various cell types in particular in lymphoid cells (Schimmer and Parker, 2001; Miller et al., 1998). The main response consists of inhibition of the release of pro-inflammatory cytokines (e.g. TNF-α, interleukin-1, etc.) or other pro-inflammatory molecules (Abbas et al., 2000; Schimmer and Parker, 2001).

In contrast to this classical direct anti-inflammatory action of glucocorticoids, the present effect of prednisolone, i.e. downregulation of endothelial and mesangial ecto-apyrase, seems paradoxically rather pro-inflammatory than anti-inflammatory: thus reduced ecto-apyrase of endothelial cells may lead to reduced hydrolysis of pro-inflammatory or pro-aggregatory mediators (e.g. extracellular ATP or ADP, respectively). Therefore, it seems that downregulation of this ecto-enzyme may promote rather than inhibit the presence of these pro-inflammatory nucleotides.

However, prevention of Hx activation due to decreased ecto-apyrase activity mediated by prednisolone may be considered as an important anti-inflammatory activity of this drug. Thus, whereas the classical anti-inflammatory action of glucocorticoids may be directed predominantly towards lymphoid cells, an important additional effect of these steroids may be directed towards the ecto-apyrase of vascular and glomerular cells. Whether this latter pathway is completely or partly non-genomic, remains to be settled. The relevance of the present in vitro effect of prednisolone for the in vivo situation in subjects with corticosteroid responsive nephrotic syndrome in relapse is uncertain. It is clear that, in contrast to other forms of primary glomerular disorders, most patients with corticosteroid responsive nephrotic syndrome can be cured by treatment with prednisolone (Brady et al., 2001). The reason for this beneficial response upon prednisolone is unknown. Although glucocorticoid action through the "classical" pathway upon lymphoid cells remains probably also important in these patients, it is tempting to speculate that the unique beneficial effect of prednisolone exclusively occurring in corticosteroid responsive nephrotic syndrome may be mediated through an additional non-genomic effect of prednisolone upon endothelial and mesangial cells.

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