Differential Regulations of AQP4 and Kir4.1 by Triamcinolone Acetonide and Dexamethasone in the Healthy and Inflamed Retina

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PURPOSE. Glucocorticoids are used to treat macular edema, although the mechanisms underlying this effect remain largely unknown. The authors have evaluated in the normal and endotoxin-induced uveitis (EIU) rats, the effects of dexamethasone (dex) and triamcinolone acetonide (TA) on potassium channel Kir4.1 and aquaporin-4 (AQP4), the two main retinal Müller glial (RMG) channels controlling retinal fluid movement.

METHODS. Clinical as well as relatively low doses of dex and TA were injected in the vitreous of normal rats to evaluate their influence on Kir4.1 and AQP4 expression 24 hours later. The dose-dependent effects of the two glucocorticoids were investigated using rat neuroretinal organotypic cultures. EIU was induced by footpad lipopolysaccharide injection, without or with 100 nM intraocular dex or TA. Glucocorticoid receptor and channel expression levels were measured by quantitative PCR, Western blot, and immunohistochemistry.

RESULTS. The authors found that dex and TA exert distinct and specific channel regulations at 24 hours after intravitreal injection. Dex selectively upregulated Kir4.1 (not AQP4) in healthy and inflamed retinas, whereas TA induced AQP4 (not Kir4.1) downregulation in normal retina and upregulation in EIU. The lower concentration (100 nM) efficiently regulated the channels. Moreover, in EIU, an inflammatory condition, the glucocorticoid receptor was downregulated in the retina, which was prevented by intravitreal injections of the low concentration of dex or TA.

CONCLUSIONS. The results show that dex and TA are far from being equivalent to modulate RMG channels. Furthermore, the authors suggest that low doses of glucocorticoids may have antiedematous effects on the retina with reduced toxicity.

Macular edema (ME) results from an imbalance between increased fluid entry in the retina due to the blood-retinal barrier breakdown and/or deregulation of fluid withdrawal mechanisms that include metabolic activity of retinal Müller glial (RMG) cells, retinal pigment epithelium, and choroidal vessels. RMG cells play a major role to maintain fluid homeostasis within the retina. They span the entire thickness of the retina, establishing an anatomic and functional connection between the retinal neurons and the retinal blood vessels, on one hand, and with the vitreous and the subretinal space on the other hand.1,2 RMG cells are essential to control potassium movements, mainly through the K inward rectifying channel Kir4.1 activity. In the mammalian retina, Kir4.1 is mostly expressed in the end feet of RMG and in their processes coating the blood vessels; this polarized expression allows K influx into RMG from the extracellular spaces surrounding activated neurons and its delivery to the retinal vessels and the vitreous, a process referred to as “potassium siphoning.”3,4 Aquaporin-4 (AQP4) acts in concert with K channels to maintain osmotic homeostasis in the retina. In RMG, it is mainly located in perivascular and end foot processes, where it is thought to form a multiprotein complex involving Kir4.1 and allows water movements into and out of RMG cells.5 Water permeability is greatly reduced in null AQP4 RMG cells, highlighting the central role of AQP4 in water transport in the retina.6 However, the functional interaction between AQP4 and Kir4.1 remains controversial, since AQP4 invalidation does not affect the cellular distribution of Kir4.1 and potassium currents in Müller cells.4

AQP4 and Kir4.1 are important to control retinal swelling, and most retinal pathologies are accompanied by alterations of amounts and/or spatial distribution of AQP4 or Kir4.1. For example, in blue light–injured rat retina, AQP4 was increased in the outer retina, whereas Kir4.1 was decreased and mislocalized.5 In a rat model of branch retinal vein occlusion, both AQP4 and Kir4.1 transcripts were downregulated with a mislocalization of Kir4.1 protein.8 In rats with endotoxin-induced uveitis (EIU), Kir4.1 and AQP4 were differentially reduced.9

More than 800 studies have been published on the use of corticosteroids to treat ME of different origins.10–14 Recently, controlled studies have shown that repeated intravitreal triamcinolone acetonide (TA) injections did not result in better visual acuity than grid photocoagulation in diabetic ME.15 Dexamethasone (dex) and fluocinolone acetonide have been used in sustained release degradeable or nondegradable intravitreal implants, resulting in more controlled and constant.
drug release. Dexamethasone intravitreal implants (Ozurdex, Allergan, Irvine, CA), containing either 0.7 or 0.35 mg dex, were evaluated in a randomized study. They reduced the risk of vision loss and improved the speed and incidence of visual improvement in eyes with ME secondary to branch retinal vein occlusion or central retinal vein occlusion and have been recently approved for these indications.16

However, few studies have addressed the mechanisms of action of corticosteroids in the normal and diseased retina and it remains unknown whether different glucocorticoids may exert different actions, particularly on ME. Corticosteroids bind to the glucocorticoid receptor (GR) and to the mineralocorticoid receptor (MR), two related transcription factors that exert pleiotropic effects.17–20 We have recently shown that the retina (and particularly RMC cells) expresses the GR and also the MR; unexpectedly we found that the MR ligand aldosterone increases the expression of AQP4 and Kir4.1 and induces retinal swelling.21 Whether and how glucocorticoids also regulate these channels are open questions, inasmuch as these drugs are currently injected in the vitreous to treat ME. The hallmark of these treatments in clinical practice is the use of high doses of corticosteroids that may have considerably undesirable side effects, such as glaucoma and cataract.22–23

To progress in the rationale of use of corticosteroids in ophthalmology, we have evaluated the effects of intraocular injections of clinical as well as relatively low doses of two glucocorticoids, dex and TA, on the expression and localization of AQP4 and Kir4.1, in the normal rat retina and in EIU, a model of acute intraocular inflammation causing retinal swelling. We show that dex and TA are not equivalent in regulating either AQP4 or Kir4.1.

**Methods**

**Animals**

All experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and European Communities Council Directive 86/609/EEC. Adult female Lewis rats (8–12 weeks old; Janvier, Le Genest-Saint-Ise, France) were used for retinal organotypic cultures and for in vivo experiments. Anesthesia of rats was induced by intramuscular injection of ketamine (100 mg/kg; Virbac, Carros, France) and chlorpromazine hydrochloride (0.65 mg/kg; Largactil, Sanofi Aventis, Livron sur Drome, France). Rats were killed by carbon dioxide inhalation.

**Rat Retinal Explant**

After dissection of the neuroretina from the posterior part of the rat eye, the neuroretinal organotypic cultures were prepared as previously described.21 They were incubated in steroid-free media for 24 hours and then treated with 10 nM to 1 μM dex and TA (Sigma-Aldrich, Saint Quentin Fallavier, France) for a further 24 hours to evaluate their dose-dependent effects. The glucocorticoids were dissolved in steroid-free media containing 0.1% ethanol. Control retinas were treated with 0.1% ethanol in media.

**Table 1. Real-Time PCR Primers**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Species</th>
<th>SYBR Green Primers</th>
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<tr>
<td><strong>18S</strong></td>
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<td>Sense</td>
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<td><strong>Kir4.1</strong></td>
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**AQP4 and Kir4.1 Regulation by Glucocorticoids**

Intravitreous Injections in Normal Rat Eyes

Intravitreous injections were performed using microfine (300 μL) syringes with 30G needles under topical anesthesia (tetracaine 1%; Aldrich, Lyon, France). We used clinical doses of dex (1 μM final concentration in the vitreous, which is about the concentration expected after implantation of 700 μg of dex in a slow release polymeric system; Ozurdex) and TA (40 μg/μL, which corresponds to 9 μM final in the vitreous), and a lower dose (100 nM) for both steroids. Control rat eyes were injected with vehicle (0.9% NaCl); rats were killed 24 hours after injections. The neuroretinas were then removed for real-time PCR, immunohistochemistry, retinal flat mounting, and Western blot analyses, respectively.

**EIU Induction and Glucocorticoid Prevention**

EIU was induced in rats by a single footpad injection of 100 μL sterile pyrogen-free saline containing 200 μg lipopolysaccharide (LPS) from Salmonella typhimurium (Sigma-Aldrich) as previously described.24 Intravitreous injections of 100 nM dex or TA were performed in the rat eyes at the time of LPS challenge. Normal rat eyes and eyes from EIU rats without intravitreous injection and with vehicle injections were used as controls. After 24 hours, rats were killed and neuroretinas retrieved for real-time PCR and immunohistochemistry analyses.

**Reverse Transcription and Real-Time PCR**

Total RNA was isolated from treated neuroretinal explants and rat retinas from normal injected and EIU rat eyes (RNeasy Plus Mini Kit; Qiagen, Courtaboeuf, France). First-strand cDNA was synthesized using random primers (Invitrogen, Cergy Pontoise, France) and reverse transcriptase (Superscript II; Invitrogen). Transcript levels of GR, Kir4.1, and AQP4 were analyzed by real-time PCR performed in a real-time PCR system (7500 Real-Time PCR System; Applied Biosystems, Foster City, CA) with fluorescent DNA binding dye (SYBR Green; Invitrogen) detection. The 18S was used as an internal control. Table 1 shows sequences of primers. Delta cycle threshold calculation was used for relative quantification of results.

**Immunofluorescence and Immunohistochemistry**

Kir4.1 and AQP4 were visualized by immunofluorescence, as described previously,21 using rabbit anti-Kir4.1 (1:200; Alomone Laboratories, Jerusalem, Israel) and rabbit anti-AQP4 (1:200; Millipore, St. Quentin en Yvelines, France) antibodies. GR was detected by immunohistochemistry using rabbit anti-GR (1:2000; Santa Cruz Biotechnology, Heidelberg, Germany) antibody as described.21

**Retinal Flat Mounting**

Eyes were fixed for 15 minutes in 4% paraformaldehyde (Ladd Research Industries, Inland Europe, Conflans-sur-Lanterne, France). After being washed, retinas were isolated, cut by four orthogonal incisions, and postfixed with acetone 100% at −20°C for 15 minutes. They were then rehydrated with PBS containing 1% Triton X-100, and incubated with the rabbit anti-AQP4 antibody (1:100) at room temperature under stirring overnight. After being washed with PBS, the Alexa Fluor 488–conjugated goat anti-rabbit IgG (1:100; Molecular Probes, Leiden, The Netherlands) was used as a secondary. After washing, retinas were mounted on slides in DAPI stain, a fluorescent DNA binding dye (Invitrogen) education. The affixes were used as an internal control. Table 1 shows sequences of primers. Delta cycle threshold calculation was used for relative quantification of results.
Netherlands) was applied for 1 hour. Blood vessels were stained with TRITC-labeled lectin from Bandeiraea simplicifolia B8-1 (1:100; Sigma-Aldrich). The retinas were flat-mounted using gel mount. Images were taken using a confocal laser scanning microscope (Zeiss LSM 710; Carl Zeiss Microimaging, Oberkochen, Germany).

Western Blot Analysis

Rat retinas were homogenized in NP40 buffer in the presence of protease inhibitors. Equal amounts of protein (20 μg) were separated on 4–12% Tris-glycine gel (Novex; Invitrogen), transferred to nitrocellulose, and the blots were incubated overnight with primary antibodies at 4°C. The membranes were washed, incubated with horseradish peroxidase–conjugated goat anti-rabbit IgG (1:3000; Vector, AbCys, Paris, France) for 1 hour at room temperature, and developed using Western blotting detection reagents (ECL Plus; GE Health Care, Orsay, France). The β-actin was used as an internal control. The following primary antibodies were used: rabbit anti-Kir4.1 (1:400), rabbit anti-AQP4 (1:750), and rabbit anti-β-actin (1:1000; Abcam, Cambridge, UK).

Statistics

Data were expressed as mean ± SE. Statistical analysis was made using a software program for predictive analytics (GraphPad Prism5; GraphPad Software, San Diego, CA). A one-way ANOVA test followed by Bonferroni’s comparison was used. Mann–Whitney test was used when two groups were compared. Values of P < 0.05 were considered significant.

RESULTS

Dex but Not TA Upregulates Kir4.1 in the Normal Rat Retina

When clinical (high) doses of glucocorticoids (dex 1 μM and TA 40 μg/μL) were injected in the normal rat eyes, dex increased the Kir4.1 protein level in the neuroretina, and TA was ineffective (Fig. 1A). However, both dex and TA differentially changed the spatial distribution of Kir4.1 protein. Dex induced a diffuse increase from the Müller end feet, to perivascular processes, and up to their apices. (Fig. 1B). Immunofluorescence of rat retinas shows that dex significantly upregulates Kir4.1 mRNA (quantitative RT-PCR) at 1 μM, whereas TA has no significant effect. (D) Intravitreous injection of lower dose (100 nM) of dex and TA has no significant effect on Kir4.1 mRNA levels (quantitative RT-PCR) in normal rat retinas. The 18S is used as housekeeping gene. Results are relative values compared with sham-treated controls. Data are expressed as mean ± SE, n = 4 retinas per condition. Statistical significance: **P < 0.01.
processes, and up to their apices (Fig. 1B, c–d). TA on the
other hand concentrated Kir4.1 in the Müller end feet and around retinal vessels (Fig. 1B, e–f).

In rat retinal explants, Kir4.1 transcripts were significantly upregulated by dex at 1 μM, and TA had no effect (Fig. 1C), showing that at all tested doses, TA did not significantly influence Kir4.1 expression. In vivo injection in the vitreous using lower dose (100 nM) dex or TA did not significantly modify Kir4.1 mRNA expression (Fig. 1D).

**TA but Not Dex Downregulates AQP4 in the Normal Rat Retina**

Western blot analysis of rat retinas from eyes injected with clinical doses of dex and TA showed that TA (not dex) significantly reduced AQP4 protein content in the neuroretina (Fig. 2A). AQP4 distribution was also differently affected by dex and TA, as shown by immunohistochemistry analysis. Although TA decreased AQP4 in RMG end feet and around vessels (Fig. 2B, m–n), dex enhanced perivascular recruitment of AQP4 (Fig. 2B, g–h). Confocal imaging of flat-mounted retinas from rats treated in vivo allowed a further analysis of AQP4 distribution. Dex enhanced the perivascular distribution of AQP4 in the superficial- and intraretinal capillary plexus (Fig. 2B, i–l), whereas TA had the opposite effect (Fig. 2B, o–r).

Dose dependence was evaluated on retinal explants treated ex vivo. Under these conditions, AQP4 mRNA expression was decreased by TA at all tested doses from 10 nM to 1 μM, whereas dex marginally reduced it (Fig. 2C). Intravitreous injection of lower dose of glucocorticoids (100 nM) confirmed that AQP4 transcripts are negatively regulated by TA, but not dex (Fig. 2D).

**EIU and Glucocorticoids Affect GR Expression in the Rat Retina**

After 24 hours of LPS challenge, a time when EIU is at its highest intensity in our model, GR mRNA was significantly downregu-

![AQP4 expression in rat retinas 24 hours after dex and TA treatments.](image)
lated in the retina compared with normal rats (Fig. 3A). In ganglion cells and in cells of the inner nuclear layer (where the nuclei of RMG are located), the immunolabeling nuclear signal was also reduced (Fig. 3D).

A single injection of 100 nM dex or TA prevented the LPS-induced downregulation of GR transcripts (Fig. 3B) as well as protein expression, as shown by immunohistochemistry (Figs. 3E, 3F).

**Dex and TA Differentially Regulate Kir4.1 in EIU**

Parallel to the GR expression, Kir4.1 transcripts were significantly downregulated 24 hours after LPS challenge (Fig. 4A). Immunostaining showed only a faint labeling of Kir4.1 in the Müller cell end feet and around the vessels (Fig. 4C, c–d). Intravitreous injection of 100 nM dex significantly prevented the EIU-induced Kir4.1 mRNA decay, whereas the same dose of TA had no effect (Fig. 4B). The 18S is used as housekeeping gene. Results are relative values compared with normal rat retinas or to retinas from rats with LPS challenge and intravitreous sham injection. Data are expressed as mean ± SE, n = 4 retinas per condition. Statistical significance: *P < 0.05, **P < 0.01.

**Dex and TA Differentially Regulate AQP4 in EIU**

EIU did not significantly reduce AQP4 mRNA expression at the level of the whole neuroretina (Fig. 5A), but immunostaining of AQP4 showed that EIU induced loss of Müller cell end feet and perivascular distribution of AQP4 (Fig. 5C, c–d). In EIU conditions, 100 nM TA could significantly upregulate AQP4 expression, whereas the effect of dex did not reach statistical significance (Fig. 5B). Moreover, TA was more efficient than dex to concentrate the AQP4 immunofluorescence signal in the Müller end feet and around the vessels within the inflamed retina (Fig. 5C, e–h). In addition, TA induced a displacement of AQP4 toward the apical microvilli of the Müller cells (Fig. 5C, g–h).

**FIGURE 3.** Effects of EIU and glucocorticoids on GR expression in rat retinas. Twenty-four hours after initiation of EIU, GR (A) transcripts (quantitative RT-PCR) are significantly downregulated in the retina. Separate experiments show that a single intravitreous injection of 100 nM dex and TA prevents the decay of GR (B) mRNA. The 18S is used as housekeeping gene. Results are relative values compared with normal rat retinas or to retinas from rats with LPS challenge and intravitreous sham injection. Data are expressed as mean ± SE, n = 4 retinas per condition. Statistical significance: *P < 0.05, **P < 0.01. Immunohistochemistry of normal rat retinas shows GR (C) localization in ganglion cells and in cells in the INL. EIU reduces the GR (D) protein. A single intraocular dose of 100 nM dex (E) or TA (F) prevents the decrease of GR in the inflamed retinas. IPL, inner plexiform layer; OPL, outer plexiform layer. Scale bar, 50 μm.

**FIGURE 4.** Differential regulation of Kir4.1 by dex and TA in retinas with EIU. (A) LPS stimulates a significant decline in Kir4.1 mRNA (quantitative RT-PCR) at 24 hours. (B) Intravitreous injection of 100 nM dex prevents the downregulation of Kir4.1 transcripts 24 hours after LPS challenge. However, the same dose of TA has no significant effect. The 18S is used as housekeeping gene. Results are relative values compared with normal rat retinas (A) or to retinas from EIU rats with intravitreous sham injection (B). Data are expressed as mean ± SE, n = 4 retinas per condition. Statistical significance: *P < 0.05, **P < 0.01. (C) Immunofluorescence of Kir4.1 in rat retinas with EIU shows a faint labeling in Müller cell end feet and around retinal vessels (c, d). Although 100 nM dex increases Kir4.1 in Müller end feet and perivascular processes (e, f), the same dose of TA slightly enhances Kir4.1 in Müller end feet and OLM (g–h, open arrowheads). (b, d, f, h) Merged images of Kir4.1 (green) and DAPI (blue). Filled arrowheads: Müller cell end feet; arrows: perivascular processes. Scale bar, 100 μm.
homeostasis,20 and synthetic glucocorticoids represent major therapeutic tools as anti-inflammatory and immunosuppressive drugs (among other effects). Benefits of corticosteroids are often associated with deleterious or undesirable events, and elucidation of their tissue-specific effects should improve their therapeutic use. The wide use of corticosteroids in ophthalmology contrasts with the limited information available on their target genes and signaling pathways within the healthy and diseased retina. In retinal pathologies, high doses of TA or dex are currently injected into the vitreous cavity, and it is assumed that relatively lower concentrations reach the retinal cells, due to low solubility. Toxicity and side effects may result in part from the use of high doses,22,25,26 and an important issue is to establish whether lower doses could be efficient.

Glucocorticoids bind to the GR to regulate transcriptional activity of target genes; they can also bind to and activate the MR. Permanent occupancy of the MR by circulating glucocorticoids is prevented to a large extent by the metabolizing enzyme 11-beta hydroxysteroid dehydrogenase type II (HSD2) in mineralocorticoid-sensitive tissues that coexpress MR and HSD2, since 11-dehydro (or -keto) metabolites of natural glucocorticoids (as 11-dehydro corticosterone in rodents, or cortisone in humans) are inactive on MR.17–19,27 Noticeably, these metabolites are also inactive on the GR, raising the question of the extent of GR activation in vivo in cells with GR and high expression of HSD2, as kidney distal nephron, or retinal cells.21 At variance with metabolites of natural glucocorticoids, the 11-ketoDex is a specific and potent GR agonist: in HEK (human embryonic kidney) cells, it was shown that 11-ketoDex was as active as cortisol on hGR transactivation activity, whereas cortisone was fully inactive; TA was not evaluated in this study.28 Thus, dex should be much more efficient than natural glucocorticoids to transactivate the GR, even in cells with high HSD2 expression.

In this study, we have addressed the questions of dex and TA activities, after in vivo intravitreous injections in normal and pathologic retina, and we have selected as targets two channels that control retinal hydration, Kir4.1 and AQP4. Our results clearly show that the two glucocorticoids have distinct and complex effects. On the whole, it appears that dex mainly regulates Kir4.1 (not AQP4), whereas the reverse is true for TA (AQP4, not Kir4.1). Dex increases Kir4.1 expression both in normal and in inflamed retina. A striking feature of TA action is its capacity to downregulate AQP4 in normal retina and its opposite effect in uveitis.

The differential gene regulations reported here are evident at concentrations much lower than those in clinical ophthalmology, suggesting that low doses of glucocorticoids may be efficient (and could limit their toxicity) for therapeutic goals. Indeed, when GR are transfected in host cells with reported genes, their transcriptional activity is elicited at low glucocorticoid concentration. Half-maximal transactivation activity (EC50) of human GR expressed in CV1 cells occurred at 0.6 nM for dex and at 3.4 nM for TA.29 However, in primary human ocular trabecular meshwork cells,30 dex and TA exhibited much closer EC50 (3.0 vs. 1.5 nM), underlining the influence of the cell context. Of note, TA is also a weak agonist on human MR (MR), given that its EC50 value is close to 300 nM, compared with dex (5 nM) and aldosterone (0.05 nM).29,31,32 We have previously shown that in the normal rat retina, Kir4.1 is regulated through both GR and MR pathways, whereas AQP4 is more likely a specific mineralocorticoid target in RMG cells.21 With the doses used in this study, dex may activate both MR and GR, leading to upregulation of Kir4.1, whereas GR activation by TA decreases AQP4 expression in healthy retina.

EIU, originally used as a model of anterior uveitis, involves inflammation of the ocular posterior segment accompanied by retinal edema.35 We observed a striking downregulation of GR in the retina of EIU rats, which was not reported before. Similarly, GR was found to be downregulated by LPS in rat hippocampus36; GR and MR were both reduced in mouse brain microglia under LPS challenge.37 MR (not GR) has been identified as a downregulated gene in human choroidal but not in

**FIGURE 5.** Differential regulation of AQP4 by dex and TA in retinas with EIU. (A) LPS does not reduce significantly AQP4 mRNA at 24 hours (quantitative RT-PCR). (B) Intravitreous injection of 100 nM TA but not dex upregulates AQP4 transcripts in inflamed retinas. The 18S is used as housekeeping gene. Results are relative values compared with normal rat retinas (A) or to retinas from EIU rats with intravitreous sham injection (B). Data are expressed as mean ± SE, n = 4 retinas per condition. Statistical significance: *P < 0.05. ns, no significance. (C) Immunofluorescence of AQP4 in rat retinas with EIU shows a loss of AQP4 localization in Müller cell end feet and around retinal vessels (a, b). A single intravitreous injection of 100 nM TA (g, h) is more effective than dex (e, f) to restore AQP4 in Müller end feet and perivascular processes. TA induces an additional staining toward the apices of Müller cells (g, open arrowhead). (b, d, f, h) Merged images of AQP4 (green) and DAPI in (blue). Filled arrowheads: Müller cell end feet; arrows: perivascular processes. Scale bar, 100 μm.

**DISCUSSION**

Corticosteroids induce a wide diversity of cellular responses that depend on the cell context, the nature of the steroid, and the interacting pathways.25 They are key hormones to maintain homeostasis,20 and synthetic glucocorticoids represent major therapeutic tools as anti-inflammatory and immunosuppressive drugs (among other effects). Benefits of corticosteroids are often associated with deleterious or undesirable events, and elucidation of their tissue-specific effects should improve their

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**AQP4 expression in EIU**

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retinal endothelial cells in a transcriptomic study of EIU. The resistance of SPRET/Ei mice to LPS stimulation was abrogated by GR antagonism, suggesting that a decrease in GR expression may favor LPS response. Interestingly, we also show that intraocular glucocorticoids can prevent the EIU-induced downregulation of GR. Although corticosteroid receptor levels are little influenced by their ligand concentrations in physiological conditions, this may not be the case in a pathologic context.

We found a significant downregulation of Kir4.1 at 24 hours after the LPS challenge, confirming an earlier report; in both studies, AQP4 downregulation was less marked than that for Kir4.1. In addition, the loss of perivascular localization of AQP4 in RMG cells reported here in EIU was not observed by Liu and colleagues. In the context of inflamed retina, dex and TA also exert distinct regulation of Kir4.1 and AQP4; however, TA appears as a positive regulator of AQP4, an effect opposite to that observed in normal retina. This may relate to the interactions of the glucocorticoid and the inflammatory pathways. The complexity of AQP4 involvement in pathologic situations is also illustrated by the consequences of its genetic invalidation. Although AQP4 deletion has been shown to be protective in cytotoxic brain edema, it aggravates vasogenic brain edema by reducing water clearance from brain parenchyma.

We showed here relatively short-term effects of glucocorticoid occurring 24 hours after intravitreous injection, a situation that may differ somehow from the long-term action of these drugs, as used for therapeutic objectives. If the difference between dex and TA holds true for a long time, the two glucocorticoids should have different pharmacologic outcomes on retinal functions, leading to different indications in retinal pathology.

In conclusion, we have shown that the two main glucocorticoids used in clinical ophthalmology, dex and TA, exert differential regulations of AQP4 and Kir4.1, indicating that they are not functionally equivalent. Furthermore, their control of channel expression levels can be evidenced at doses much lower than those used in clinics; we propose that these lower doses may be therapeutically active on the retina while limiting adverse effects.

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References

