The Glucocorticoid Triamcinolone Acetonide Inhibits Osmotic Swelling of Retinal Glial Cells via Stimulation of Endogenous Adenosine Signaling

Ortrud Uckermann, Franziska Kutzera, Antje Wolf, Thomas Pannicke, Andreas Reichenbach, Peter Wiedemann, Sebastian Wolf, and Andreas Bringmann

Paul Flechsig Institute of Brain Research (O.U., T.P., A.R.), Interdisziplinäres Zentrum für Klinische Forschung (O.U., A.W.), and Department of Ophthalmology and Eye Clinic (F.K., P.W., A.B.), University of Leipzig Medical Faculty, Leipzig, Germany; and Department for Ophthalmology, University Bern, Inselspital, Bern, Switzerland (S.W.)

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ABSTRACT

The glucocorticoid triamcinolone acetonide is clinically used for the treatment of macular edema. However, the edema-resolving mechanisms of triamcinolone are incompletely understood. Since cell swelling is a central cause of cytotoxic edema in the brain and retina, we determined the effects of triamcinolone acetonide on the swelling of retinal ganglion and Müller glial cells in acutely isolated retinas from rats and guinea pigs in situ. Triamcinolone acetonide (100 μM) had no effect on the swelling of ganglion cells that was evoked in isolated whole mounts of the guinea pig retina by acute application of glutamate (1 mM) or high K⁺ (50 mM). However, triamcinolone reversed the osmotic swelling of Müller glial cells in retinas of the rat that was observed under various experimental conditions: in retinas isolated at 3 days after transient retinal ischemia, in retinas of eyes with lipopolysaccharide-induced ocular inflammation, and in control retinas in the presence of Ba²⁺ (1 mM), H₂O₂ (200 μM), arachidonic acid (10 μM), or prostaglandin E₂ (30 nM). The inhibiting effect of triamcinolone on osmotic glial cell swelling was mediated by stimulation of transporter-mediated release of endogenous adenosine and subsequent A₁ receptor activation, resulting in an elevation of the intracellular cAMP level and activation of the protein kinase A, and, finally, in an opening of extrusion pathways for K⁺ and Cl⁻ ions. The inhibitory effect on the cytotoxic swelling of glial cells may contribute to the fast edema-resolving effect of vitreal triamcinolone observed in human patients.

Cystoid macular edema is an important complication of various oculocutaneous diseases such as diabetic retinopathy and retinal vein occlusion. In patients with uveitis or diabetic retinopathy, macular edema is the major cause of severe visual deterioration. Although the precise cause of cystoid macular edema is still uncertain, inflammatory and ischemic-hypoxic conditions have been causally implicated in the development of retinal edema (Tao, 1982; Yanoff et al., 1984; Guex-Crosier, 1999; Marmor, 1999). Extracellular (vasogenic) fluid accumulation, as a main causative factor of tissue swelling and cyst formation, occurs after breakdown of the blood-retina barriers (Gass et al., 1985) induced by vascular endothelial growth factor (VEGF) (Antcliff and Marshall, 1999; Marmor, 1999) and/or inflammatory mediators (Derevjanik et al., 2002). Vascular leakage results in serum protein extravasation and osmotically driven water inflow into the retinal tissue. In addition to this vasogenic mechanism, water accumulation within retinal neurons and glial cells (resulting in cell swelling, i.e., cytotoxic or intracellular edema) may contribute to the edema in the ischemic and postischemic retina (Pannicke et al., 2004; Uckermann et al., 2004b). During diabetic retinopathy, the swelling of ganglion cell bodies and their processes precedes the loss of these cells and the glosis of the inner retinal layers (Duke-Elder and Dobree, 1967). There are good arguments for a contribution of glial (Müller) cell swelling to the development of cystoid
macular edema, e.g., in cases without significant angiographic vascular leakage (Fine and Brucker, 1981; Yanoff et al., 1984). A similar situation has been described for the brain where the postischemic or traumatic edema is characterized by swelling of astroglial cells (Kimelberg, 1995). In the retina, dysregulated fluid-absorbing mechanisms normally carried out by pigment epithelial and glial cells (Bringmann et al., 2004) may contribute to edema formation, resulting in an ineffective redistribution of fluid from the retina into the blood. The observation that clinically significant diabetic macular edema occurs only when (in addition to vascular leakage) also the active transport mechanisms of the blood-retinal barriers are dysfunctional (Mori et al., 2002) underlines the importance of disturbed fluid clearance in the development of retinal edema.

Anti-inflammatory corticosteroids are commonly used to treat inflammatory eye diseases, and among them, triamcinolone acetonide (9α-fluoro-16α-hydroxyacetprednisolone) has the advantage to form crystals that represent local depots for the sustained release over relatively long periods of time. Intravitreal triamcinolone is used clinically for the rapid resolution of macular edema (Ip et al., 2003; Massin et al., 2004). However, the mechanisms by which triamcinolone exerts its fast edema-resolving effect are incompletely understood. There is evidence that triamcinolone inhibits vasogenic edema and inflammation. Triamcinolone decreases vascular leakage (Ando et al., 1994), reduces the secretion of VEGF by pigment epithelial cells during oxidative stress (Matsuda et al., 2005), down-regulates the expression of the VEGF gene in vascular smooth muscle cells (Nauck et al., 1998), and reduces the vitreal level of VEGF in patients with diabetic retinopathy (Brooks et al., 2004). Furthermore, triamcinolone decreases the paracellular permeability of cultured epithelial cells, down-regulates the inflammatory expression of endothelial adhesion molecules (Penfold et al., 2000), and inhibits leukocyte-endothelial interactions in the diabetic retina of the rat (Tamura et al., 2005). However, it is not known whether triamcinolone, in addition to its inhibitory action on vasogenic edema, also has an effect on the development of cytotoxic (intracellular) edema. Inhibition of cytotoxic edema may contribute to the rapid edema-resolving effect of triamcinolone observed in human patients. Therefore, we investigated whether triamcinolone inhibits the swelling of retinal neurons or glial cells. Neuronal cell swelling was evoked by application of glutamate or high K⁺ onto acutely isolated whole mounts of the guinea pig retina, which is known to rapidly induce swelling of retinal neurons by activation of ionotropic glutamate receptors, resulting in intracellular Na⁺ and Cl⁻ overload and water influx (Uckermann et al., 2004b). Müller cell swelling was investigated in slices of the rat retina during exposure of a hypotonic solution (a situation that resembles hypoxia-induced cytotoxic edema in the brain). We investigated the osmotic swelling of retinal glial (Müller) cells during various experimental conditions: in a rat model of transient retinal ischemia reperfusion, in a model of ocular inflammation induced by intravitreal injection of lipopolysaccharide (LPS), during acute application of the K⁺ channel blocker Ba²⁺ or of arachidonic acid and prostaglandin E₂ (PGE₂), respectively, and during oxidative stress induced by acute application of H₂O₂.

Materials and Methods

Materials. Mitotracker Orange (chloromethyltetramethylrhodamine) was purchased from Molecular Probes (Eugene, OR). Ketamine was obtained from Ratiopharm (Ulm, Germany) and xylazine from BayerVital (Leverkusen, Germany). PGE₂ was delivered from Calbiochem (San Diego, CA), and Ly341495 was from Tocris Cookson Inc. (Bristol, UK). Triamcinolone acetonide, acetazolamide, arachidonic acid, 4-hromophenacyl bromide, indomethacin, 8-cyclopentyl-1,3-dipropylnoxinol (DPCPX), 8-(3-chlorostyryl) caffeine (CSC), N(6)-cyclopentadenedenosine (CPA), 2-methyl-6-(phenylethyl)-piperidine (MPEP), bis-(o-aminophenoyloxide)-N,N,N',N'-tetra-acetic acid acetoxyethyl ester (BAPTA/AM), 8,4-chlorophenyllthio) (pCPT)-CAM, forskolin, 3-isobutyl-1-methanxine (IBMX), 2,3-dideoxyadenosine, H-89, flufenamic acid, 5-nitro-2-(3-phenylo-plyaminobenzoic acid (NPPB), N-nitrobenzylxinosine (NBTi), ARL-67156, adenosine-5′-O-(a,b-methylene)-diphosphonate (AOPCP), and all other substances used were purchased from Sigma Chemical (Deisenhofen, Germany).

Animal Models. All experiments were carried out in accordance with applicable German laws and with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Adult Long-Evans rats (250–350 g) were used to induce transient retinal ischemia in one eye of the animals; the other eye remained untreated and served as control. Anesthesia was induced with intramuscular ketamine (100 mg/kg) and xylazine (5 mg/kg). The anterior chamber of the treated eye was cannulated from the pars plana with a 27-gauge infusion needle, connected to a bag containing normal saline. The intraocular pressure was increased to 160 mm Hg for 60 min by elevating the saline bag. Sham treatment (i.e., using the same procedures without elevation of the saline bag) does not induce alterations of the osmotic swelling characteristics of Müller glial cells when compared with control (Pannicke et al., 2004). Ocular inflammation alters the swelling characteristics of Müller glial cells in a similar fashion as ischemia reperfusion (Pannicke et al., 2005). We used an animal model of uveoretinitis induced by intravitreal injection of LPS (Becker et al., 2000). During ketamine/xylazine anesthesia, LPS from Escherichia coli 055:B5 (0.5%; Sigma-Aldrich) dissolved in 2 μl of saline was injected into one eye of the animals. At 3 days after transient retinal ischemia or at 7 days after intravitreal injection of LPS, the animals were killed by CO₂, and the retinas were removed.

Glial Cell Swelling. To determine volume changes of Müller glial cells evoked by hypotonic stress, the cross-sectional area of Müller cell somata in the inner nuclear layer of retinal slices was measured. Acutely isolated retinal slices (thickness, 1 mm) were placed in a perfusion chamber and loaded with the vital dye Mitotracker Orange (10 μM). It has been shown that Mitotracker Orange is taken up selectively by Müller glial cells, whereas neurons remain unstained (Uckermann et al., 2004a). The stock solution of the dye was prepared in dimethyl sulfoxide (DMSO) and resolved in extracellular solution that contained 136 mM NaCl, 3 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, and 11 mM glucose, adjusted to pH 7.4 with Tris. The recording chamber was continuously perfused with extracellular solution; test substances were added by rapid changing of the perfusate. The hypotonic solution was made by adding distilled water. Ba²⁺ (1 mM) was added to the extracellular solution, and was preincubated for 10 min. H₂O₂, PGE₂, and triamcinolone were applied simultaneously with the hypotonic solution. A stock solution of triamcinolone was made by using DMSO; vehicle alone had no effect on cell volume. Blocking substances were preincubated for 10 to 45 min. All experiments were performed at room temperature. The slices were examined by using a confocal laser scanning microscope LSM 510 Meta (Carl Zeiss GmbH, Jena, Germany). Mitotracker Orange was excited at 543 nm, and emission was recorded with a 560-nm long-pass filter. During the experiments, the Mitotracker Orange-stained somata in the inner nuclear layer were recorded at...
the focal plane of their largest extension. To assure that this focal plane was precisely recorded, the cell soma investigated was continuously refocused in the course of the experiments.

**Neuronal Cell Swelling.** To investigate neuronal cell swelling, the nonvascularized guinea pig retina was used, which (in comparison with the retina of the rat) has the advantage that the cell bodies in the ganglion cell layer can be recorded without interference with blood vessels and astrocytes. The animals (550–650 g) were killed by CO₂, and the retinas were removed. Pieces of retinal whole mounts (5 mm²) were explanted and mechanically fixed in a perfusion chamber, with their vitreal surface up. The whole mounts were loaded with Mitotracker Orange resolved in extracellular solution. Elevation of the K⁺ concentration was generated by equimolar reduction of Na⁺. Images were taken at two focal planes: the ganglion cell and inner plexiform layers (Fig. 1A). Reflected light was acquired by using a 650-nm HeNe laser and a 340-nm long-pass filter.

**Data Analysis.** To determine the extent of glial cell swelling, the cross-sectional area of Mitotracker Orange-stained cell bodies in the inner nuclear layer of retinal slices was measured manually using the image analysis software of the laser scanning microscope. The values are expressed in percentage of the control data measured before iso- or hypotonic challenge (100%). To determine the extent of neuronal cell swelling, two parameters were estimated as described previously (Uckermann et al., 2004b): the cross-sectional area of neuronal cell bodies in the ganglion cell layer, as well as the cross-sectional area of Müller cell fibers that pass through the inner plexiform layer; a decrease of the fiber area is caused by swelling of synapses structures located in this layer. Data are expressed as means ± S.E.M. Statistical analysis was made by using the Prism program (GraphPad Software Inc., San Diego, CA); significance was determined by Mann-Whitney U test or by analysis of variance followed by comparisons for multiple groups. Curve fits were made by using the Boltzmann equation.

**Results**

**Neuronal Cell Swelling.** The swelling of neuronal cells was investigated in whole mounts of the guinea pig retina. It is known that acute application of glutamate (1 mM) to the whole mounts causes rapid swelling of neuronal cell bodies in the ganglion cell (by ~30%; P < 0.001) (Fig. 1B) and inner nuclear layers (not shown) (Uckermann et al., 2004b). Simultaneously, the glial (Müller) cell fibers in the inner plexiform layer decrease their thickness (by ~30%; P < 0.001) (Fig. 1C), caused by swelling of synaptic structures. A similar neuronal cell swelling can be observed during application of a high-K⁺ (50 mM) solution to the acutely isolated retinal whole mounts, as well as in the ischemic guinea pig retina just after reperfusion (Uckermann et al., 2004b). The high-K⁺-evoked neuronal cell swelling is mediated by stimulation of endogenous glutamate release and subsequent activation of ionotropic glutamate receptors (Uckermann et al., 2004b).

To investigate a possible effect of triamcinolone on neuronal cell swelling, the cross-sectional areas of neuronal cell bodies in the ganglion cell layer and of Müller cell fibers in the inner plexiform layer were recorded. Extracellular application of triamcinolone acetonide (100 µM) did not decrease the magnitude of neuronal cell swelling in the ganglion cell layer evoked either by glutamate (1 mM) or by high K⁺ (Fig. 1D). Likewise, the glutamate- or high-K⁺-evoked shrinkage of the glial cell profiles in the inner plexiform layer was not altered by triamcinolone (Fig. 1E). The data suggest that acute application of triamcinolone does not inhibit neuronal cell swelling.

![Image](https://example.com/image.png)
Gliar Cell Swelling. As shown earlier (Pannicke et al., 2004), the somata of Müller glial cells in slices of 3-day postischemic retinas responded to acute hypotonic stress by an increase in their cross-sectional area by 10 to 20%, whereas cell somata in control retinas did not increase their size (Fig. 2A). A similar osmotic soma swelling was observed in retinal slices isolated at 7 days after intravitreal application of LPS (Fig. 2D) (Pannicke et al., 2005), as well as in control retinas when the K⁺ channels were blocked by extracellular Ba²⁺ ions (Fig. 3A). The osmotic swelling of the cells in diseased retinas has been shown to be caused by the down-regulation of distinct K⁺ channels, which impairs the extrusion of K⁺ ions under hypotonic conditions and can be mimicked by closure of K⁺ channels in control cells in the presence of the K⁺ channel blocker Ba²⁺ (Pannicke et al., 2004).

Although triamcinolone (100 μM) had no effect on the size of glial cell soma when the slices of 3-day postischemic rat retinas were perfused with an isotonic extracellular solution, it fully inhibited the soma swelling evoked by perfusion of a hypotonic solution (P < 0.001) (Fig. 2, A and B). In contrast, acetazolamide (100 μM), a carbonic anhydrase blocker that is used clinically to resolve macular edema, did not reverse the osmotic swelling of glial cell soma in postischemic retinas (Fig. 2C). Triamcinolone (100 μM) also inhibited the osmotic swelling of glial cell soma in slices obtained at 7 days after intravitreal application of LPS (Fig. 2D), as well as in control retinas in the presence of Ba²⁺ (1 mM) (Fig. 3B). The inhibitory effect of triamcinolone on the Ba²⁺-evoked hypotonic glial cell swelling was dose-dependent (Fig. 3C). The concentration estimated to evoke half-maximal inhibition was 1.3 μM. Vehicle (DMSO) alone had no effect (Fig. 3F).

Oxidative stress is a major cause of retinal injury after ischemia (Osborne et al., 2004). To determine whether oxidative stress induces swelling of glial cell soma, slices of control rat retinas were perfused with H₂O₂. As shown in Fig. 3D, H₂O₂ did not induce swelling of glial cell soma at isotonic conditions. However, hypotonic challenge evoked soma swelling in the presence (but not in the absence) of H₂O₂. The swelling-inducing effect of H₂O₂ was dose-dependent, with a half-maximal effect at 4 μM (Fig. 3D, inset). When triamcinolone (100 μM) was applied simultaneously with the hypotonic solution, the soma swelling evoked by H₂O₂ was fully inhibited (Fig. 3E).

It is known that arachidonic acid evokes vasogenic and cytotoxic brain edema (Chan et al., 1983) and swelling of cultured astrocytes (Staub et al., 1994). Metabolites of arachidonic acid such as prostaglandins (especially PGE₂) are implicated in the development of retinal edema during ocular inflammation. To determine whether arachidonic acid or PGE₂ may cause glial cell swelling, both substances were tested acutely in slices of control rat retinas. Arachidonic acid (Fig. 4A) and PGE₂ (Fig. 4B) evoked swelling of glial cell soma when the substances were applied simultaneously with the hypotonic solution. An involvement of endogenous arachidonic acid and PGE₂ in the osmotic soma swelling in postischemic retinas is suggested by the blocking effects of the inhibitor of the phospholipase A₂, 4-bromophenacyl bromide (Fig. 4, C and D), and of the cyclooxygenase inhibitor, indomethacin (Fig. 4, E and F), respectively. Triamcinolone inhibited the soma swelling evoked by arachidonic acid or PGE₂ (Fig. 4, A and B).

Mechanism of the Inhibitory Effect of Triamcinolone on Glial Swelling. It is known that adenosine (which is rapidly released within the retina upon ischemia; Roth et al., 1997) exerts a protective effect against ischemic injury in the retina by the activation of A1 receptors (Larsen and Osborne, 1996; Ghiardi et al., 1999). A swelling-inhibiting effect of adenosine may contribute to this protective effect in ischemic injury. To determine whether adenosine inhibits the osmotic swelling of glial cell soma, we applied adenosine simultaneously with the hypotonic solution to retinal slices. Adenosine inhibited the osmotic swelling of glial cell soma in postischemic retinas (Fig. 5A) and in control retinas in the presence of extracellular Ba²⁺ (Fig. 5B) or arachidonic acid (Fig. 5C). This effect of adenosine was mediated by activation of A1 receptors since a selective A1 receptor agonist, CPA, also inhibited the swelling, and since the effect of

**Fig. 2.** Effect of triamcinolone on the osmotic soma swelling of Müller glial cells in slices from 3-day postischemic retinas and from endotoxin-treated eyes, respectively. Swelling was induced by changing the perfusate into a hypotonic solution (60% of control osmolarity). A, hypotonic challenge resulted in soma swelling in the case of postischemic retinas, whereas Müller cells in control retinas did not increase the cross-sectional area of their soma. Application of triamcinolone (100 μM) simultaneously with the hypotonic solution fully inhibited the soma swelling. The insets show original records of two dye-filled soma in a postischemic retina, before (left) and during (right) exposure of hypotonic medium. Scale bar, 5 μm. B and C, mean cross-sectional area of Müller cell soma in slices of postischemic retinas, in dependence on the recording conditions. D, mean soma area in retinal slices derived from eyes at 7 days after intravitreal injection of LPS. Triamcinolone (100 μM) or acetazolamide (100 μM) were applied simultaneously with the iso- or hypotonic solution, as indicated. The values were measured after 4-min perfusion with the iso- or hypotonic solution and are expressed as percentage of the control value measured before application of the test solution (100%). The bars represent values obtained in 4 to 19 cells. Significant differences versus control (100%): *, P < 0.05; **, P < 0.01; ***, P < 0.001. Significant blocking effects: †, P < 0.05; ††, P < 0.001.
Adenosine was blocked by the selective antagonist of A1 receptors, DPCPX (Fig. 5B). In contrast, the swelling-inhibiting effect of adenosine was not modified by a selective A2a receptor antagonist, CSC (Fig. 5B).

To determine whether triamcinolone inhibits the osmotic swelling of glial cell somata via stimulation of the release of endogenous adenosine and subsequent stimulation of adenosine receptors and transporters, theophylline, a blocker of A1 and A2 receptors, fully reversed the effect of triamcinolone (Fig. 6A). To determine the subtype of adenosine receptors that mediates the swelling-inhibiting effect of triamcinolone, selective antagonists of A1 and A2a receptors, DPCPX and CSC, respectively, were tested. As shown in Fig. 6B and C, DPCPX fully blocked the swelling-inhibiting effect of triamcinolone, whereas CSC had no effect. On the other hand, MPEP and LY341495, antagonists of subtype 5 of groups I and II metabotropic glutamate receptors, did not block the effect of triamcinolone (Fig. 6B). The data suggest that the effect of triamcinolone is mediated by a release of endogenous adenosine and subsequent activation of A1 receptors. Thus, we tried to determine whether this adenosine was released from intracellular compartments via stimulation of nucleoside transporters or generated by degradation.
of extracellular ATP. For this purpose, various blocking substances were tested. The inhibitor of nucleoside transporters, NBTI, blocked the effect of triamcinolone (Fig. 6D). On the other hand, the ecto-ATPase inhibitor, ARL-67156, and the ectonucleotidase inhibitor, AOPCP, did not block the swelling-inhibiting effect of triamcinolone (Fig. 6E). These data suggest that the effect of triamcinolone was mediated by stimulation of transporter-mediated release of adenosine from intracellular compartments but not by extracellular formation of adenosine via degradation of ATP. Tetrodotoxin failed to interfere with the effect of triamcinolone (Fig. 6F), suggesting that it is independent of the activation of retinal neurons.

Finally, we tried to identify the intracellular signaling mechanisms that inhibit swelling after A1 receptor stimulation. The swelling-inhibiting effects of triamcinolone and of adenosine remained unaltered in the presence of the membrane-permeable Ca²⁺/H⁺ chelator BAPTA/AM (Fig. 7A and B), suggesting that an intracellular Ca²⁺/H⁺ response was not involved in the effects of both agents. On the other hand, application of a cAMP-enhancing cocktail that contained pCPT-cAMP (a membrane-permeable cAMP analog), forskolin (a direct activator of adenylyl cyclase), and IBMX (a phosphodiesterase inhibitor), reduced the osmotic glial soma swelling by approximately two-thirds in postischemic retinas and in control retinas in the presence of Ba²⁺ (1 mM) (Fig. 7C). To explore whether the effects of triamcinolone and adenosine were mediated by an activation of the adenylyl cyclase and a subsequent activation of protein kinase A, blockers of both enzymes were tested. 2,3-Dideoxy-
adenosine, an inhibitor of the adenylyl cyclase, blocked the effect of triamcinolone (Fig. 7E). Likewise, an inhibitor of the protein kinase A, H-89, reduced the effects of triamcinolone (Fig. 7E) and adenosine (Fig. 7F) by approximately two thirds.

Arachidonic acid has been shown to inhibit the efflux of osmolytes such as amino acids and Cl\(^-\) from cultured astrocytes, an effect that may contribute to osmotic swelling (Sanchez-Olea et al., 1995). Thus, the inhibition of cell swelling may involve the opening of extrusion pathways for osmolytes. To determine whether adenosine inhibits osmotic swelling by opening of such extrusion pathways for K\(^+\) and/or Cl\(^-\), the K\(^+\) channel blocker, quinine, and the Cl\(^-\) channel blockers, fluafenamic acid and NPPB, were tested. Indeed, these substances were found to block the effect of adenosine (Fig. 7G). Taken together, our data suggest that the inhibitory effect of triamcinolone on osmotic Müller cell swelling involves the release of endogenous adenosine and subsequent activation of A1 receptors. A1 receptor activation results in enhancement of the intracellular cAMP level and activation of the protein kinase A, which finally causes a compensatory efflux of K\(^+\) and Cl\(^-\) ions.

**Discussion**

Various ischemic and inflammatory ocular diseases are associated with the development of macular edema that may be caused by vascular leakage (vasogenic, extracellular edema) and/or by swelling of retinal cells (cytotoxic, intracellular edema). It has been suggested that swelling of Müller glial cells contributes to macular edema (e.g., in cases without significant angiographic vascular leakage; Fine and Brucker, 1981; Yanoff et al., 1984) and that a dysregulation of retinal fluid absorption, normally carried out by pigment epithelial and Müller glial cells (Bringmann et al., 2004), is likely to be crucial for the formation of chronic edema (Mori et al., 2002). Since Müller cells mediate the water transport between the inner retinal tissue and the blood and vitreous (Bringmann et al., 2004), Müller cell swelling, when it occurs in vivo, may reflect such a dysregulated fluid clearance.

It has been shown that experimental ischemia reperfusion of the rat retina evokes a biphasic water accumulation in the retina; the retinal water content increases strongly during the ischemic episode and increases again slowly within days after reperfusion (Stefansson et al., 1987). The edema that
develops during the ischemic period is likely caused by neuronal cell swelling and is mediated, at least in part, by overstimulation of ionotropic glutamate receptors, which allows strong ion fluxes into retinal neurons, accompanied by water uptake (Uckermann et al., 2004b). The edema that develops after reperfusion may be caused, at least in part, by glial cell swelling (Pannicke et al., 2004). A similar pattern of neuronal and glial cell edema was described in the ischemic retina of the rabbit; during ischemic episodes, both plexiform layers and the cytoplasm of neuronal cells become edematous, whereas the Müller glial cells appear to be unaffected. However, in the postischemic tissue, the Müller glial cells become edematous, whereas neural elements degenerate (Johnson, 1974).

Experimental ischemia reperfusion or endotoxin-evoked ocular inflammation cause a significant alteration of the osmotic swelling characteristics of Müller glial cells in the rat retina (Pannicke et al., 2004, 2005). During hypertonic stress, Müller cells in posts ischemic retinas (Fig. 2, A and B) or in retinas obtained from eyes displaying ocular inflammation (Fig. 2D) increase their soma volume, whereas cells in control retinas do not swell (Fig. 2A). The experimental model of acute hypertonic challenge used in the present study may be representative for pathological in vivo conditions. Hypotonic challenge mimics the osmotical conditions at the glio-vascular and glio-vitreal interfaces during pathological states, such as ischemia, in which the retinal parenchyma becomes hyperosmotic in comparison with the blood and vitreous. When the Müller glial cells take up excess osmolytes (e.g., K⁺ and Na⁺/glutamate during periods of pathological neuronal hyperecitation) and are impaired in their ability to redistribute these osmolytes into the blood and vitreous, an in-
creased intracellular osmolarity may constitute an osmotical driving force for water movement from the blood and vitreous into the Müller cells, resulting in cell swelling (Pannicke et al., 2004). However, the experimental model of hypotonic stress used in the present study may also reflect the conditions at the glio-synaptic interface since it is known that glial uptake of excess $K^+$ causes a reduction of the extracellular osmolarity (Dietzel et al., 1989). Ion fluxes from the extracellular space into the synapses through ionotropic receptors may contribute to the fall of extracellular osmolarity during neuronal activity. Therefore, experimental hypotonic stress may mimic the osmotic gradients during neuronal hyperexcitation that occurs during ischemic insults.

The present results suggest that inflammatory mediators and/or oxidative stress (that is one factor causing neuronal degeneration during reperfusion; Osborne et al., 2004) mediate the alteration of the osmotic swelling characteristics of Müller glial cells. Müller cell swelling in the postsischemic retina may be induced by inflammatory mediators, due to the activation of phospholipase A$_2$ and cyclooxygenase by osmotic stress. The mechanisms how arachidonic acid, PGE$_2$, or $H_2O_2$ induce hypotonic glial cell swelling are unclear. It has been shown that arachidonic acid inhibits distinct outwardly directed $K^+$ currents in isolated Müller cells (Bringmann et al., 1998), and it is conceivable (but remains to be proven) that arachidonic acid and PGE$_2$ close plasma membrane pathways that are involved in osmolyte extrusion out of the cells. A failure in osmolyte extrusion upon hypotonic challenge would cause water movement into the cells and, thus, cell swelling. The observation that blockade of $K^+$ and Cl$^-$ channels inhibits the effect of adenosine (Fig. 7G) strongly supports the view that a closure of ion release pathways is involved in the induction of osmotic swelling. It is known that arachidonic acid induces both vasogenic and cytotoxic edema in the brain (Chan et al., 1983; Wahl et al., 1993) and evokes swelling of cultured astrocytes (Staub et al., 1994), and it has been suggested that the swelling-inducing effect of arachidonic acid is mediated by a direct inhibition of the efflux of osmolytes such as amino acids and Cl$^-$ (Sanchez-Olea et al., 1995).

Corticosteroids such as triamcinolone are used clinically to resolve macular edema (Ip et al., 2003; Massin et al., 2004). The mechanisms of the triamcinolone effects are incompletely understood; present research is focused on the inhibition of vascular edema. It has been shown recently that triamcinolone induces a very rapid reduction of macular edema in patients with retinal vein occlusion and diabetic retinopathy, which is evident as early as 1 h after treatment (Miyamoto et al., 2005). However, the regulation of tight junctional and VEGF protein expression by triamcinolone is suggested to occur at the gene expression level that needs several hours. Therefore, it has been suggested that triamcinolone acts, at least additionally, via activation of signaling cascades (Miyamoto et al., 2005). Here, we present data supporting the idea that triamcinolone inhibits Müller cell swelling in situ via stimulation of endogenous adenosine signaling. A1 receptor stimulation after release of endogenous adenosine causes an activation of the adenylyl cyclase and of the protein kinase A that results in a stimulation of compensatory ion release by Müller glial cells. The lack of effects of inhibitors of the ecto-ATPase and the ectonucleotidase on the effect of triamcinolone (Fig. 6E) suggests that triamcinolone selectively stimulates the transporter-mediated release of adenosine from retinal cells, whereas it has no effect on the extracellular formation of adenosine via degradation of ATP. It is conceivable that triamcinolone stimulates the water clearance function of Müller cells in situ by the activation of ion secretion via Müller cell end feet into the blood and vitreous. Similar rapid effects of triamcinolone (within minutes) have been described for other cell systems, e.g., for the remodeling of the nuclear envelope structure in Xenopus oocytes (Shahin et al., 2005). Our results suggest that stimulation of A1 receptors by adenosine results in an enhancement of the intracellular cAMP level. Although in most cell systems activation of A1 receptors causes a decrease of the cAMP level, there are observations that (in dependence on the density of the receptors expressed by the cells) A1 receptors may couple also to stimulating G proteins, resulting in an enhancement of the intracellular cAMP level (Cordeux et al., 2000). However, the inhibitory effect of cAMP elevation on cell swelling was only partial (Fig. 7, C and D). Similarly, blockade of the adenylyl cyclase or of the protein kinase A resulted in a partial inhibition of the effects of triamcinolone and adenosine (Fig. 7, E and F), suggesting that a second intracellular pathway may contribute to the signaling from A1 receptors to ion channels.

In the present study, we used triamcinolone in a concentration of 100 μM (i.e., 43 μg/ml), unless stated otherwise. In human patients, a vitreal dosage of 1 mg/ml is used regularly (4 mg of triamcinolone in 4 ml of human vitreous volume), and peak concentrations between 2 and 7 μg/ml in aqueous humor samples were described to occur within days after a single 4 mg intravitreal injection of triamcinolone (Beer et al., 2003). We observed a half-maximal effect of triamcinolone at 1.3 μM, i.e., 0.56 μg/ml (Fig. 3C), which is well in the range of concentration observed in human subjects.

In summary, we show that triamcinolone does not inhibit glutamate- and high-$K^+$-evoked swelling of retinal neurons but inhibits the osmotic swelling of Müller glial cells during retinal ischemia reperfusion and inflammation. This inhibitory effect of triamcinolone on cytotoxic glial cell swelling may contribute to the fast edema-resolving effect of this substance observed in human patients. This view is supported by the observation that triamcinolone also rapidly resolves cystoid macular edema in patients without significant angiographic vascular leakage. Inhibition of glial cell swelling may represent also a component of the protective effect of corticosteroids used in the treatment of brain edema.

References


Triamcinolone Inhibits Glial Cell Swelling


Address correspondence to: Dr. Andreas Bringmann, Department of Ophthalmology and Eye Clinic, University of Leipzig, Liebigstrasse 10-14, D-40103 Leipzig, Germany. E-mail: bria@medizin.uni-leipzig.de