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

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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 ocular 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 gliosis of the inner retinal layers (Duke-Elder and Doobree, 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 posts ischemic or traumatic edema is characterized by swelling of astrogial 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-hydroxydiprenisolone) 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 ocul ar 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.
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 acetone (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.

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**Fig. 1.** Acute application of glutamate or high-K⁺ evokes morphological alterations in whole-mount guinea pig retinas that are not inhibited by triamcinolone. The effects of glutamate (1 mM) and high (50 mM) K⁺ were determined after 10 and 5 min of exposure, respectively. Triamcinolone (100 μM) was applied either alone or simultaneously with glutamate or high K⁺. A, in an acutely isolated retinal slice, Mitotracker Orange (10 μM) selectively stained Müller glial cells (green). The morphological alterations were recorded in whole mounts at two focal planes. GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; NFL, nerve fiber layer; ONL, outer nuclear layer; OPL, outer plexiform layer. B, application of glutamate (1 mM) caused swelling of neuronal cell bodies in the GCL. The Müller cell end feet are green-stained, and the unstained neuronal cell bodies reflect light (red). Yellow-appearing elongated structures are nerve fiber bundles. C, green-stained Müller cell profiles in the IPL decreased their thickness in response to glutamate; the synaptic structures reflect red light. Scale bars, 20 μm. D, mean cross-sectional area of the neuronal cell bodies in the GCL. E, mean cross-sectional area of the Müller cell profiles in the IPL. The bars represent values obtained in 20 to 103 cells. Significant differences versus control: *, P < 0.001.
Glial 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 somata 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 somata in postischemic retinas (Fig. 2C). Triamcinolone (100 μM) also inhibited the osmotic swelling of glial cell somata 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 somata, slices of control rat retinas were perfused with H₂O₂. As shown in Fig. 3D, H₂O₂ did not induce swelling of glial cell somata 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 somata 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 somata, we applied adenosine simultaneously with the hypotonic solution to retinal slices. Adenosine inhibited the osmotic swelling of glial cell somata 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
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.

**Fig. 3.** Triamcinolone inhibits the osmotic soma swelling of Müller glial cells in slices of control retinas. The swelling was induced by changing the perfusate into a hypotonic solution (60% of control osmolarity) in the presence of extracellular Ba$^{2+}$ (1 mM) or H$_2$O$_2$. A, in the presence of Ba$^{2+}$, hypotonic challenge evoked a reversible swelling of Müller cell somata in control retinas. The mean cross-sectional area of glial cell somata was measured in dependence on the recording time. B, triamcinolone (100 μM) inhibited the osmotic swelling of Müller cell somata in the presence of Ba$^{2+}$. Mean cross-sectional area of the somata in dependence on the recording conditions. C, dose dependence of the inhibiting effect of triamcinolone on the Ba$^{2+}$-evoked osmotic soma swelling. The curve was fitted with the Boltzmann equation and a half-maximal inhibitory concentration of 1.3 μM. D, H$_2$O$_2$ evoked dose-dependently soma swelling under hypotonic conditions. Inset, data points were fitted with the Boltzmann equation, with a half-maximal effect at 4 μM. E, triamcinolone (100 μM) inhibited the osmotic soma swelling in the presence of H$_2$O$_2$ (200 μM). F, vehicle (DMSO; 0.1%) had no effect on the soma swelling in the presence of Ba$^{2+}$ (1 mM) or H$_2$O$_2$ (200 μM). Triamcinolone was applied simultaneously with the iso- or hypotonic solutions. B to F, values were measured after 4-min perfusion of the iso- or hypotonic solution and are expressed as percentage of the control value measured before application of the solution (100%). The bars represent values obtained in 4 to 45 cells. Significant difference versus control (100%): *, P < 0.05; **, P < 0.01; ***, P < 0.001. Significant blocking effect: †, P < 0.05; ††, P < 0.01; †††, P < 0.001.
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 Ca\(^{2+}\)/H\(^{+}\) chelator BAPTA/AM (Fig. 7, A and B), suggesting that an intracellular Ca\(^{2+}\)/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 (Fig. 7C) and in control retinas in the presence of Ba\(^{2+}\) (1 mM) (Fig. 7D). 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, flufenamic 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 hypotonic 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 hypotonic 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 hyperexcitation) and are impaired in their ability to redistribute these osmolytes into the blood and vitreous, an in-
increased 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 postischemic retina may be induced by inflammatory mediators, due to the activation of phospholipase A₂ and cyclooxygenase by osmotic stress. The mechanisms how arachidonic acid, PGE₂, or H₂O₂ 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 PGE₂ 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 signifying 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


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