

Electrophysiological Effects of Corticosteroids on the Retinal Pigment Epithelium

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PURPOSE. As corticosteroids appear to intervene in pathogenesis of central serous chorioretinopathy, ion transport changes within the retinal pigment epithelium (RPE) might be involved. Electrophysiological responses to corticosteroid administration were recorded in vivo and in vitro.

METHODS. *Clinical study:* The standing ocular potential was recorded during intravenous (IV) infusion of glucose 5% and glucose 5% + prednisolone 0.2% in 14 patients with relapsing multiple sclerosis. The results were compared with a control group receiving two successive identical glucose 5% infusions. *In vitro study:* Native tissue explants (RPE + choroid, porcine, and bovine) were placed in a Ussing-type chamber. After baseline determination of the transepithelial potential (PD), short circuit current (I_{sc}) and transepithelial resistance (R_t), the effect of apical hydrocortisone (HC) 10^{-4} M was determined.

RESULTS. *Clinical study:* A significant rise of the standing potential was found after glucose infusion ($P = 0.005$), whereas no change was detected after IV glucose + prednisolone ($P = 0.695$). *In vitro study:* In the porcine RPE, the mean baseline PD and I_{sc} were significantly reduced (both $P = 0.012$) after applying apical 10^{-4} HC. R_t was also significantly reduced ($P = 0.01$). The same type of response, observed in bovine RPE, was reduced in low chloride/low bicarbonate conditions.

CONCLUSIONS. Corticosteroids modified electrophysiological parameters representing RPE function in vivo. The existence of an RPE-specific effect was confirmed in vitro. Further work is required to link the observed ion transport changes to a reduction of apical, subretinal fluid absorption. (*Invest Ophthalmol Vis Sci.* 2001;42:472-475)

Retinal pigment epithelial (RPE) cells form a monolayer that regulates the transport of fluids, ions, and metabolites between the sensory retina and the vascular choroidal system. The RPE constitutes the outer part of the blood-retinal barrier. An impairment of this barrier, as it is observed in central serous chorioretinopathy, presents with subretinal fluid accumulation. As demonstrated by indocyanine green angiography, fluid accumulation is currently thought to be linked to vascular abnormalities within the choriocapillaris.^{1,2} However, it is

probable that RPE dysfunction is equally associated, thus leading to localized breakdown of the outer blood-retinal barrier.

Currently, there is some evidence for pathophysiological links between corticosteroid treatment and the incidence of central serous chorioretinopathy.³⁻⁶ The existence of this hypothesized effect at the RPE level has never been demonstrated clearly. Fluid transport changes across an intact RPE are related to transepithelial ion flux, which necessarily induce measurable electrophysiological changes. The purpose of this study was to demonstrate the existence of RPE-related electrophysiological effects of corticosteroids in vivo and in vitro.

MATERIALS AND METHODS

Clinical Study

Patients. Healthy volunteers were recruited among the staff of Lille University Hospital (Lille cedex, France). Patients with multiple sclerosis without any history of visual impairment were included in the study. The research followed the tenets of the Declaration of Helsinki; informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study. The patients were scheduled for treatment with systemic corticosteroids (1 g of prednisolone during 4 hours) for relapsing multiple sclerosis. A clinical ophthalmologic examination was performed on all patients, evaluating the best corrected visual acuity and oculomotricity; fundus examination by indirect ophthalmoscopy was also performed. Patients with ocular motility palsy, clinical evidence of optic neuritis (visual acuity < 20/25, optic disc pallor), or uveitis were not included. In all patients, the recordings were initiated between 1:30 and 2:15 PM. All patients had finished their meals by 12:30 PM at the latest.

Recording of the Standing Potential of the Eye in the Prednisolone Group. In a preliminary study, the standing ocular potential was recorded in the group of 14 healthy controls. Skin electrodes were placed in accordance with ISCEV guidelines to perform electro-oculography.⁷ The subject was placed in Ganzfeld conditions at a luminance of 60 cd/m² for 10 minutes. Then the recording procedure started. Every 2 minutes, during a 58-minute period, the standing potential of the eye was recorded as the subject performed target-triggered saccades. The analysis of the mean standing potential of the right eye revealed a slow oscillation response with a mean periodicity of 34 minutes (Fig. 1). The first trough occurred at 12 minutes, the second at 46 minutes. The shift between the minimum (trough) to the maximum amplitude (peak) was determined. This shift was found to be statistically significant (Table 1).

In the patients with multiple sclerosis, a similar procedure was used. However, the procedure was split in two recording sessions of 24 minutes each (phase 1 and 2), to allow the patient to take a rest for 10 minutes, remaining in the same light-adapted conditions. The first intravenous infusion (80 drops/min) was initiated at $t = 11$ minutes (phase 1) and the second at $t = 45$ minutes (phase 2). The duration of both infusions was 13 minutes.

The first group of consecutive patients was assigned to the prednisolone group. They first received (at $t = 11$ minutes) an intravenous infusion of glucose 5% and then (at $t = 45$ minutes) an intravenous infusion of glucose 5% and prednisolone 0.2%. It was estimated that the plasma level of 100 μ M was obtained within 10 minutes.⁸ The

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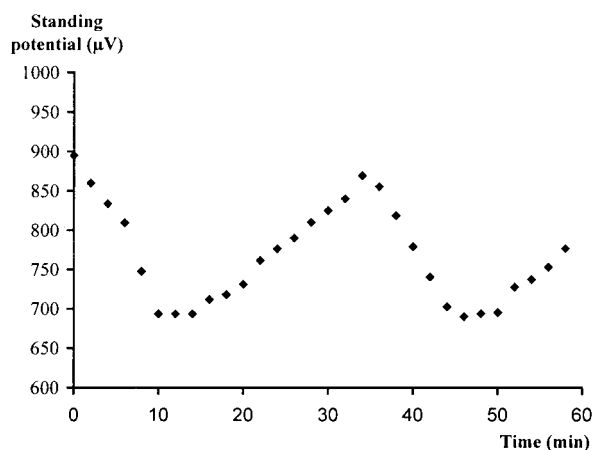


FIGURE 1. Recording of the mean standing potential of the right eye in 14 healthy volunteers with no infusion.

second group of patients was assigned to the glucose group, in which only two successive infusions of glucose 5% were compared, and no prednisolone was added to the second infusion.

Statistics. Results are shown as means \pm SD. Only the recording of the right eye of each patient was analyzed. Comparisons of paired sets of data (trough to peak shift) within each group of subjects (phase 1 versus phase 2) were made using a Wilcoxon rank test. Comparisons of unpaired sets of data (trough-to-peak shift) between the different groups of subjects (phase 1: glucose versus prednisolone, phase 2: glucose versus prednisolone) were made using a Mann-Whitney *U* test. For both series of tests, $P < 0.05$ was considered significant.

In Vitro Tissue Study

Retinal Pigment Epithelium Preparations. The research followed the tenets of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Large white pigs were enucleated after premedication with intramuscular ketamine and general anesthesia with pentobarbital. Bovine eyes were provided by a local slaughterhouse. The eyes were transferred to phosphate-buffered saline (composition in mM/l: NaCl 140, KCl 4, Na₂HPO₄ 0.5, KH₂PO₄ 0.15 [pH 7.4]).

The eyes were opened posterior to the limbus (3 mm in porcine eyes, 6 mm in bovine eyes) with a surgical blade, then cut on the entire circumference with surgical scissors. The choroid was separated from the sclera by blunt dissection. The retina was then gently removed from the underlying RPE.

In Vitro Electrophysiological Study. The native tissue (pigment epithelium + choroid) was placed between two half-Ussing-type chambers within 4 hours after enucleation. A 0.163 cm² area of the tissue sample was exposed to 10 ml of solution on each side (apical side = retina and basolateral side = choroid) and gassed with 12%

O₂-5% CO₂-83% N₂, as low oxygen increases longevity of RPE function.⁹ The experiments were conducted at 37°C. The short-circuit current (*I*_{sc}) was monitored continuously using a DVC 1000 voltage clamp (WPI, Aston, UK), and the potential difference (PD) was measured every 2 minutes. Voltage-sensing electrodes and the current passing bridges consisted of 3 M KCl-agar; the reference electrodes were placed at the basolateral side. Transepithelial resistance (*R*_t) was determined by clamping the PD to +10 mV at 15-second intervals, recording the deflection of *I*_{sc}, and applying Ohm's law. The preparations were allowed to equilibrate. Stabilization of bioelectrical parameters was achieved within 30 to 40 minutes. Only tissue samples with an *R*_t > 80 Ω · cm² were considered for analysis. Basal bioelectrical activity was monitored for 15 minutes before the addition of the reagents. Hydrocortisone (10⁻⁴) was dissolved in the KBR solution (vehicle) and added to the apical or the basolateral bath. This concentration was chosen in accordance with the estimated plasma and vitreous levels in the clinical part of this study.^{8,10} Changes in PD, *I*_{sc}, and *R*_t were calculated as the variation between the values measured immediately before the addition of reagents and the values corresponding to the peak phase.

Solutions. Krebs-Ringer bicarbonate buffer (KRB) had the following composition (in mM/l): 140 Na, 120 Cl, 5.2 K, 1.2 Mg, 1.2 Ca, 2.4 HPO₄, 0.4 H₂PO₄, 25 HCO₃, 5.5 glucose, and 1.0 glutathione (pH 7.3).

Cl-free solution was obtained by replacing NaCl and KCl by Na and K gluconate salts. In the bicarbonate-free solution, NaHCO₃ was replaced with NaGluconate.

Statistical Analysis. Results are expressed as means \pm SD for each set of preparations. Comparisons of paired sets of data (PD, *I*_{sc}, *R*_t, respectively before and after addition of HC) were made using a Wilcoxon rank test.

Comparisons of unpaired sets of data (PD, *I*_{sc}, *R*_t) corresponding to different bath solutions (KRB versus Cl-free solution, Cl-free solution versus Cl-free solution + bumetanide, Cl-free solution versus Cl-free-HCO₃-free solution) were made using a Mann-Whitney *U* test. For both series of tests, $P < 0.05$ was considered significant.

RESULTS

Clinical Study

The first 14 consecutive patients were included in the prednisolone group. The evolution of the mean standing potential is shown in Figure 2. Before IV infusion of glucose, the standing potential of the eye decreased. After starting the IV infusion of glucose (phase 1), it increased as it did in healthy volunteers with no infusion (Fig. 1). The rise of the standing potential was found to be less pronounced after infusion of glucose + prednisolone (phase 2), compared with the phase 1 response (obtained with the glucose infusion). The phase 1 rise reached statistical significance at $t = 20, 22,$ and 24 minutes. The phase 2 rise of the standing potential after infusion of glucose +

TABLE 1. Effect of Intravenous Prednisolone on the Standing Potential of the Eye

Infusion	Prednisolone		Glucose		Control	
	Glucose	Glucose + Prednisolone	Glucose 1	Glucose 2	Control 1	Control 2
Trough (μ V) $t + 2$ min	672 \pm 205	682 \pm 230	653 \pm 137	656 \pm 163	697 \pm 137	693 \pm 206
Peak (μ V) $t + 14$ min	755 \pm 242	704 \pm 276	711 \pm 175	721 \pm 188	776 \pm 206	776 \pm 208
Wilcoxon	$P = 0.005$	$P = 0.695$	$P = 0.003$	$P = 0.001$	$P = 0.009$	$P = 0.016$
Mean Difference Peak/ Trough (μ V)	83	22	58	65	79	83
Wilcoxon	$P = 0.001$		$P = 0.689$		$P = 0.765$	

Values are mean \pm SD. Data are from the right eye of the 14 patients and 14 controls. The statistical significance of difference between paired sets of data was determined using a Wilcoxon rank test.

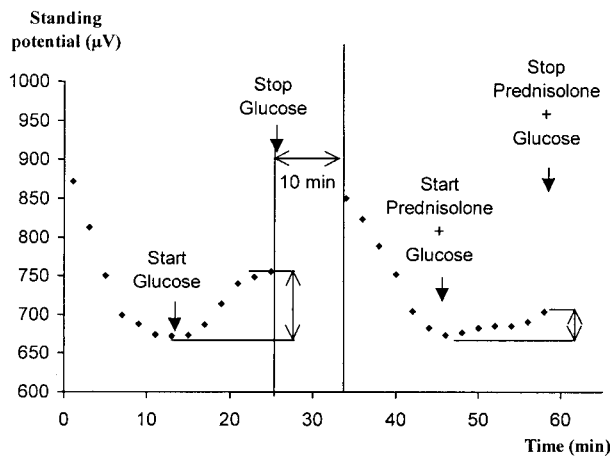


FIGURE 2. The effect of glucose versus glucose + prednisolone on the mean standing potential of the right eye of 14 multiple sclerosis patients (prednisolone group).

prednisolone never reached statistical significance. Table 1 summarizes the effect of intravenous prednisolone on the standing potential of the eye and on the peak-to-trough shift.

In the prednisolone group, comparing the peak-to-trough shift after glucose infusion (phase 1) with the peak-to-trough shift after glucose + prednisolone infusion (phase 2), the shift was found to be higher after glucose infusion in 12 out of 14 patients.

The following 14 patients were included in the glucose group. No significant difference of the standing potential of the eye could be found between the two recording sessions (Fig. 3). The peak to trough shift was found to be statistically significant in both sessions (phase 1 and phase 2); it was slightly higher after the second glucose infusion, however this difference did not reach statistical significance (Table 1).

The observed difference of the trough-to-peak shift between the prednisolone group and the glucose group did not reach statistical significance, in either phase 1 or 2 of the recording procedure.

Bovine In Vitro Study

In the bovine RPE, Table 2 summarizes the effects of HC in KRB, in low chloride and low chloride-low bicarbonate solutions. Apical 10^{-4} HC reduced the transepithelial potential, the short circuit current, and the transepithelial resistance in the KRB bath. When using the low chloride solution, the HC-mediated reduction of PD, I_{sc} , and R_t appeared to be inhibited, although not completely blocked. Adding apical bumetanide 10^{-4} , a selective inhibitor of transepithelial chloride flux, to the low chloride bath, did not significantly modify the hydro-

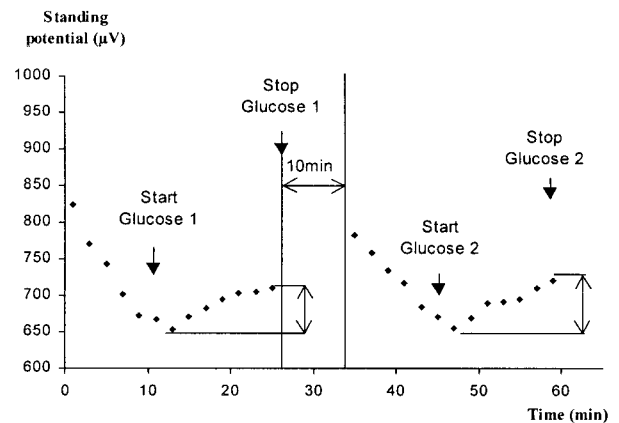


FIGURE 3. The effect of two successive glucose perfusions on the mean standing potential of the right eye of 14 multiple sclerosis patients (control group).

cortisone response, when comparing it with the response observed in low chloride solutions without bumetanide. In a bicarbonate-free-low chloride bath, the response to hydrocortisone appeared to be completely blocked.

Porcine In Vitro Study

In 8 of 20 porcine eyes, the basal electrophysiological recordings were stable, and R_t was sufficient to conduct the procedure. In eyes with low R_t , the previous blunt dissection separating the choroid from the sclera appeared to be difficult. Table 2 summarizes the effects of hydrocortisone on the electrical parameters of the porcine adult RPE. Apical hydrocortisone reduced the PD, I_{sc} , and R_t significantly (Table 2). Basolateral hydrocortisone alone produced no measurable change in PD, I_{sc} , or R_t (not shown).

DISCUSSION

The results of the present study are compatible with an inhibitory effect of corticosteroids on RPE fluid absorption in vivo and in vitro. However, it is crucial to stress the difference between the current experimental setup, which evaluated a short-term response, and the clinical presentation of central serous chorioretinopathy, which represents a chronic disease.

The electro-oculogram (EOG) is known to be one of the most appropriate ways of studying the function of human RPE,⁷ which compares the light response to the dark trough after dark adaptation. However, the light response is thought to be generated by the interaction of retina and RPE, which seems to be essentially linked to increased basolateral Cl con-

TABLE 2. Effect of Hydrocortisone on Bioelectrical Parameters

	<i>n</i>	PD Base	PD HC	<i>P</i>	I_{sc} Base	I_{sc} HC	<i>P</i>	R_t Base	R_t HC	<i>P</i>
Bovine										
KRB	7	6.0 ± 0.4	4.7 ± 0.4	0.018	55.7 ± 1.1	34.3 ± 0.8	0.018	100.9 ± 17.7	79.6 ± 17.8	0.028
Cl-free	8	3.6 ± 1.4	2.9 ± 1.0	0.012	31.0 ± 1.3	27.5 ± 1.1	0.018	134.2 ± 19.6	94.0 ± 22.6	0.123
Cl-free + Bumetanide	5	3.3 ± 1.5	3.0 ± 0.8	0.103	25.2 ± 0.8	22.1 ± 0.6	0.042	134.7 ± 23.2	128.6 ± 21.8	0.043
Cl-free HCO ₃ -free	5	1.9 ± 1.0	1.7 ± 1.1	0.402	11.3 ± 0.8	11.0 ± 0.7	0.705	139.3 ± 22.3	138.9 ± 24.9	0.345
Porcine										
KRB	8	3.8 ± 2.5	1.5 ± 1.2	0.012	41.2 ± 6.9	36.0 ± 6.0	0.012	261.7 ± 46.1	230 ± 40.3	0.01

Values are mean ± standard deviation. PD (mV), potential difference; I_{sc} , short circuit current ($\mu A \text{ cm}^{-2}$); R_t , tissue resistance ($\Omega \cdot \text{cm}^2$). Data are from *n* preparation before (base) and after (HC) apical hydrocortisone exposure. Statistical significance (*P*) of difference between paired sets of data was determined using a Wilcoxon test. The effect of HC on bovine retinal pigment epithelium demonstrates a combined chloride and bicarbonate dependency.

ductance.¹¹ Only the standing potential recorded at constant luminance conditions can be considered to be representative of RPE function. For this reason, the authors chose to develop an original protocol of EOG recording. It established that corticosteroids could induce changes of the standing potential when compared with controls. However, the hypothesized RPE-related origin of this EOG finding remained to be confirmed by *in vitro* recordings.

HC was found to modify the electrical parameters of the bovine RPE. A marked decrease of PD and I_{sc} was observed after administration of HC on the apical side of the bovine RPE. No such response was observed on the basolateral side of the preparation. Certain transport mechanisms of the bovine RPE appear to be species-dependent, yet a similar response to HC in tapetum-free porcine RPE-choroid explants confirmed these findings in the present study, in conditions that might be functionally closer to human RPE. However, the experienced firm adherence between choroid and sclera made blunt dissection more difficult, thus probably altering the electrical properties of the tissue explant. This might explain the low potential difference obtained in the current porcine study, compared with bovine RPE, one of the most widely used animal models.

Physiological functions of corticosteroid hormones involve activation of intracellular receptors as well as poorly understood membrane receptors. HC is believed to diffuse freely across the cellular membrane to be linked to a cytosol receptor.¹² In the past, some neurophysiological results suggested that corticosteroids could act nongenomically and specifically through their membrane receptor on a neuronal surface.¹³ The presence of a nuclear corticosteroid receptor has been recently demonstrated in the RPE.¹⁴ However, no membrane receptor for corticosteroids, which could explain the exclusive response to apical stimulation, has yet been identified.

Although the exact cellular mechanisms of HC in the RPE remain to be determined, the present study provides evidence for chloride/bicarbonate dependency of the response induced by HC. Both Cl^- and HCO_3^- have previously been linked to transepithelial fluid transport in bovine RPE.¹⁵ However, there was no evidence for the implication of NaK2Cl cotransport in this chloride mediated response, as it remained unchanged by pretreatment with apical bumetanide.

In previously published data on bovine RPE, an increase of the PD has been linked to apical epinephrine stimulation, enhancing apical to basal chloride flux and transepithelial fluid transport, which were both inhibited by pretreatment with apical bumetanide.¹⁶ The effect of epinephrine is known to implicate intracellular Ca^{++} as a second messenger.¹⁷

The opposite effect has been associated with increased intracellular cyclic adenosine monophosphate (cAMP), inhibiting fluid absorption in bullfrog RPE¹⁸ and basolateral membrane chloride conductance in chick RPE.¹⁹ Intracellular cAMP might not be directly implicated as a second messenger in the response observed with HC in the present study, but there is some evidence for synergistic cellular effects between cAMP and corticosteroid mediated mechanisms²⁰ that remain to be demonstrated in RPE cells.

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