Ouabain-Sensitive Na-K ATPase Response in the Rabbit Iris-Ciliary Body After Lenectomy-Vitrectomy

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We studied ouabain-sensitive Na-K adenosine triphosphatase (ATPase) activities in the iris-ciliary body of rabbit eyes after lensectomy-vitrectomy. Changes in enzyme activities were quantitatively investigated in the plasma membrane of iris-ciliary body at 0 or 7 hours and at days 1, 3, 7, and 14. The specific activity of Na-K ATPase rose to significantly higher levels than the control value at 7 hours following surgery, but returned to the baseline value after 7 days. In addition, we evaluated enzyme activities after lenectomy-vitrectomy during which SF6 or silicone oil was injected. The specific activity of Na-K ATPase following the injection of SF6 or silicone oil was significantly higher than the control value at 7 hours and did not return to the normal value even after 14 days. Consensual reaction, demonstrated by increased Na-K ATPase activity, also was found in the contralateral unoperated eyes of SF6- and silicone oil-injected rabbits. The increased Na-K ATPase activity in the iris-ciliary body after experimental surgery may play an important role in restoring swollen tissues.

Ouabain-sensitive Na-K adenosine triphosphatase (ATPase) may contribute to the regulation of cell volume and the maintenance and regulation of the intracellular and extracellular ionic environment. Furthermore, it has been proposed that the secretion of aqueous humor results from active Na transport by Na-K ATPase. The ciliary body contains a high concentration of ouabain-sensitive Na-K ATPase, and the enzyme has been localized on the plasma membrane of ciliary body epithelial cells histochemically.

Mechanical trauma such as paracentesis causes breakdown of the blood-aqueous barrier in rabbits and primates. Bartels et al. and Okisaka showed that the ciliary body, particularly in the anterior pars plicata region, is a source of secondary aqueous after paracentesis.

The iris-ciliary body also is affected during vitreous surgery by mechanical trauma, prolonged intraocular irrigation, and various methods of tamponade. In this study, we evaluated changes in the iris-ciliary body through the biochemically measured activity of Na-K ATPase after experimental lenectomy-vitrectomy. We found increased specific activity of Na-K ATPase activity in the iris-ciliary body of operated and contralateral unoperated eyes after surgery. Moreover, we compared those findings with the results of lenectomy-vitrectomy during which SF6 or silicone oil was injected.

Materials and Methods

Domestic albino rabbits, weighing about 2 kg each, were obtained from a local breeder and were housed under the same conditions. Rabbits were anesthetized with an intravenous dose of xylazine and ketamine hydrochloride. A lensectomy-vitrectomy was performed using the Peyman Vitrophage 9000 in the right eye, as described by Cottingham and Forster and Kaplan and coworkers. Ringer’s lactate solution (Ringer’s lactate supplemented with 5.0 mM glucose and 18.3 mM bicarbonate) was used during the operation. After the lensectomy-vitrectomy, 30% SF6/air and silicone oil/air exchanges were performed. Air was passed through a cellulose nitrate filter (pore size = 0.02 µm; Toyo Roshi, Tokyo). Silicone oil (1000 centistokes, specific gravity 0.98) was purchased from Koken Co., Tokyo. Erythromycin ointment was applied topically to the eye immediately afterward. Postoperative examinations were made at 1 hour and at 1, 3, and 14 days. Eyes were excluded from analysis if they had hemorrhage, retinal detachment, or glaucoma postoperatively. The left eye served as the untreated control for each animal. The care and treatment of animals in this investigation were in compliance with the ARVO Resolution on the Use of Animals in Research.

The time course of the biochemical study was mea-
Fig. 1. Specific activity of Na-K ATPase in the final pellet of iris-ciliary body after surgery in experimental (closed squares) and control (open squares) eyes. Values indicate mean ± SD (n = 4). *P < 0.01 (paired t-test).

Fig. 2. The effect of SF₆ (open squares) and silicone oil (open circles) on specific activities of Na-K ATPase in the final pellet of iris-ciliary body was compared with that of vitrectomy (Ringer’s lactate plus solution, closed squares) alone. Values indicate mean ± SD (n = 4). *P < 0.01; **P < 0.05 (paired t-test).

The Na-K ATPase assay was performed at 37°C at pH 7.5, according to the methods of Mittag et al. and Chifflet et al. For the assay, the incubation time was no more than 30 min, which was in a linear range. The specific activity of Na-K ATPase was expressed in μM inorganic phosphate liberated/mg protein/hour. Ouabain was used in parallel samples, so data shown reflect differences between Na-K ATPase activity measured ± ouabain in the incubation.

For light microscopy, the enucleated eyeballs were fixed with periodate-lysine-paraformaldehyde and were embedded in paraffin. The 3-μm-thick sections were sliced and stained with hematoxylin-eosin.

Results

Figure 1 shows the specific activities of Na-K ATPase. Immediately (0 hour) following surgery, the Na-K ATPase activity corresponded to the control value. At 7 hours after surgery, the activity was 2.3 times higher than the control value. On days 1 and 3, the...
activities were 1.5 times higher than the control value. However, after day 7, activity decreased and corresponded to the control value.

The results of specific activities of Na-K ATPase on the effect of SF6 and silicone oil are summarized in Figure 2. In the beginning, the specific activity of Na-K ATPase after the injection of SF6 or silicone oil corresponded to the specific activities of Ringer's lactate plus solution. However, these increased specific activities of the enzyme did not return to the control value even after 14 days.

Consensual reaction, that is, increased Na-K ATPase specific activity, was observed in the iris-ciliary body of contralateral unoperated eyes (Fig. 3). The specific activity in unoperated eyes of silicone oil-injected rabbits was significantly higher than that of Ringer's lactate plus solution-substituted rabbits on day 1 (P < 0.01). Thereafter, it returned to the basal level. On the other hand, prolonged increase of the specific activity in the contralateral eyes of SF6-injected rabbits was observed 7 hours (P < 0.01), 3 days (P < 0.05), 7 days (P < 0.05), and 14 days (P < 0.01) after surgery.

Light microscopy showed an extensive edema and vessel dilation of the ciliary body at day 1 after lensectomy-vitrectomy (Fig. 4a). A single layer of pigmented ciliary epithelia was observed in a tip of anterior pars plicata of the ciliary body (Fig. 4b). At day 14, the edema and vessel dilation were decreased (Fig. 4c). Conversely, when SF6 injection (Fig. 4d) or silicone oil injection (Fig. 4e) was performed, the ciliary body remained edematous even after day 14. No swelling was observed in the ciliary body of the contralateral eyes on day 14 after SF6 injection (Fig. 4f) nor in the iris on day 1 after lensectomy-vitrectomy alone (Fig. 4g).

Discussion

In the rabbit, the lens is disproportionately large, and we found it technically impossible to perform a pars plicata vitrectomy without simultaneously removing the lens. Therefore, a lensectomy was included in our experiment.14 24 Technically, removing residual lens capsule and cortex completely near the ciliary body using the Peyman Vitrophage 9000 also was difficult. These remaining substances seemed to be stimuli for cellular infiltration of the iris-ciliary body after vitreous surgery.

The biochemical results of this experiment revealed remarkable changes in ouabain-sensitive Na-K ATPase activity in the rabbit iris-ciliary body, which was examined at several time intervals during the 2 weeks after the surgical procedure.

Two results confirmed that the preparation method of Mittag et al20 was reasonable for assessing Na-K ATPase response in the iris-ciliary body. First, we recovered more than 85% of Na-K ATPase activity in...
the final pellet from crude homogenate. Second, we removed blood cells through anterior ciliary arteries by a perfusion technique with homogenization buffer using enucleated, unoperated eyes and compared the specific activity of the final pellet from the iris-ciliary body with that of nonperfused eyes. We found no significant difference in specific activity between perfused and nonperfused pellets. We concluded, therefore, that with this method blood cells did not affect specific activity of Na-K ATPase of iris-ciliary body.

In the present study, we obtained markedly increased Na-K ATPase activity in iris-ciliary body. If there were some contamination of red blood cells, the specific activity of Na-K ATPase in iris-ciliary body should have been decreased because the specific activity of red blood cells is markedly lower than that of iris-ciliary body. Thus, it seems unlikely that we overestimated the Na-K ATPase response in iris-ciliary body.

As SF₆ and silicone oil are widely used intraoperatively as vitreous substitutes, we determined the effects of these materials on the same enzyme activities. Even at postoperative day 14, the specific activities of Na-K ATPase were not restored. The delayed return to control values may indicate that extensive damage occurred to the iris-ciliary body after the injection of SF₆ or silicone oil.

Durlu et al. showed that markers of Müller cells associated with glycogenolysis and gluconeogenesis, glutamate-glutamine cycle, and cytoskeletal protein metabolism were affected by the experimental lensectomy-vitrectomy. In these experiments, the widely used vitreous substitute, silicone oil, did not appreciably change these enzyme activities in retinal Müller cells when compared postoperatively with Ringer's lactate plus solution. However, in the present study, we found that silicone oil and SF₆ apparently affected Na-K ATPase activity in the iris-ciliary body. These substitutes are not inert for the iris-ciliary body cells.

Prostaglandins have been implicated in the irritative response after mechanical trauma to the eye, resulting in miosis, vasodilation, increased protein levels in the aqueous, and increased intraocular pressure. Prostaglandins applied topically to the eye result in a breakdown of the tight junctions of nonpigmented epithelium. Breakdown of the blood-aqueous barrier occurs after paracentesis of the anterior chamber. Fragmentation of the tight junctions, particularly in the anterior pars plicata region of the ciliary body, occurs after paracentesis, with subsequent leakage of plasma protein into the aqueous humor. In the present study, we observed the swollen anterior pars plicata of the ciliary body after lensectomy-vitrectomy. Substances such as prostaglandins probably affect iris-ciliary body by an irritative effect of vitreous surgery through the breakdown of blood-aqueous barrier and resulting serum protein leakage and edema. Berggren observed that the swollen ciliary processes in vitro gradually shrunk during the course of an experiment, but that the shrinkage was inhibited by ouabain. The mechanism of the Na-K ATPase response after lensectomy-vitrectomy is unknown, but increased Na-K ATPase activity in the iris-ciliary body may play an important role in the recovery of edematous tissues.

Miyake et al. reported significant disruption of the blood-aqueous barrier in the contralateral eyes of patients undergoing cataract extraction and lens implantation surgery. They demonstrated that topical indomethacin effectively inhibited the disruption of the barrier in the surgically treated eyes but did not stifle the reaction in the contralateral eyes. Kottow and Seligman also demonstrated that the consensual reaction to paracentesis in the rabbit eye was more efficiently inhibited by nerve-blocking agents than by the prostaglandin inhibitor, aspirin. We showed increased Na-K ATPase activity in the contralateral unoperated eyes of silicone oil- and SF₆-injected rabbits (Fig. 3). The mechanism of the consensual Na-K ATPase response also is unknown, but the response is still useful for assessing the effects of vitreous substitutes. Our results show that the increased Na-K ATPase activity in the contralateral unoperated eyes of silicone oil-injected rabbits remains until day 7 after surgery but that the activity in the contralateral eyes of SF₆-injected rabbits is still high on day 14. These findings indicate that vitreous substitute SF₆ stimulates more iris-ciliary body edema and Na-K ATPase activity than silicone oil.

We concluded that ouabain-sensitive Na-K ATPase activities of iris-ciliary body showed a prominent time-dependent response that might be related to environmental changes of iris-ciliary body after experimental lensectomy-vitrectomy and may play an important role in restoring the edematous tissues.

Key words: experimental vitrectomy, iris-ciliary body, Na-K ATPase, SF₆, silicone oil, consensual reaction

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