
The effect of ouabain on the hydration of the cornea

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The effect of ouabain on the hydration of the rabbit cornea has been studied. The glycoside was found to prevent the dehydration of the cornea during temperature reversal procedures and its application to the cornea in vivo results in significant corneal swelling. A comparison of the effect of ouabain on the cornea to the drug's effect on electrolyte transport systems in the tissues suggests that the cornea regulates its hydration by a transport of electrolytes, presumably across the endothelium.

The problems concerning the mechanism by which the cornea regulates its hydration have received considerable attention but still remain unsolved. In 1873 it was suggested that the endothelium and the epithelium were impermeable to water, and consequently the cornea would not swell though surrounded by the tears and aqueous humor.¹ This theory was later abandoned when it was shown that the epithelium and the endothelium were, indeed, permeable to water, and the hypothesis was advanced that water leaked into the cornea mainly across the limbus but was

withdrawn across the epithelium and the endothelium by osmotic attraction from the hypertonic tears and aqueous.²

More recently, experimental results suggesting an active dehydration have been presented.³⁻⁶

It has been shown that if the isolated eye is cooled for 24 to 48 hours at +4° C., the cornea swells considerably. Subsequent incubation for 4 to 6 hours at +37° C. results in a dehydration to almost the normal level. Since this dehydration does not take place in the presence of metabolic inhibitors,^{7, 8} or in the absence of oxygen,⁶ it was suggested that it was due to cellular activity. The deturgescence was presumed to occur across the endothelium, since considerable dehydration could be demonstrated in the absence of the epithelium. The role of the epithelium in corneal deturgescence, however, could not be completely excluded.⁸

The concept of an active transport across the limiting membranes which would result in the removal of water from the cornea was further supported by the demonstration of a negative imbibition pressure in the corneal stroma in vivo.⁹

It has not been possible to identify the

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material which is transported out of the cornea. A transport of primarily electrolytes¹⁰ or water⁶ has been suggested. Pinocytosis was also suggested.^{11, 12}

Recently, an adenosinetriphosphatase (ATPase) has been demonstrated in the crab nerve which is activated by sodium and potassium ions and is inhibited by calcium or potassium ions in high concentrations and cardiac glycosides in low concentrations. Additional studies further indicate that this enzyme is important for the active transport of sodium ions,¹⁵⁻¹⁹ and its distribution in the eye and other tissues has been reported.^{15, 17, 20-22}

The activity of this ATPase is inhibited by a cardiac glycoside, ouabain (G-strophanthin), which has been shown to inhibit the active sodium transport across the frog skin in concentrations as low as 10^{-6} M.²³

Since Na-K ATPase activity was demonstrated in both the corneal epithelium and endothelium,²⁰ the effect of ouabain on the deturgescence of the cornea was investigated.

Material and methods

Experiments in vitro. Rabbits weighing 2 to 3 kilograms were killed with an intravenous injection of sodium pentobarbital. The eyes were enucleated immediately after the death of the animal and were each transferred to a moist chamber consisting of a 100 ml. glass jar, the bottom of which was covered with cotton-wool moistened with saline. The eyes were kept in the jars and only taken out for corneal thickness determinations. The eyes were then subjected to a temperature reversal procedure consisting of refrigeration at 4° C. for 24 hours followed by warming for 4 hours at 37° C. The thickness of the cornea was determined before and after the cooling and warming periods, respectively.

In some experiments oxygen gas saturated with water was allowed to bubble through the incubation jar when the temperature was held at +37° C. There was no distinguishable difference between the experiments with or without the gas. Therefore, oxygen was omitted in about half of the experiments.

Ten drops of a ouabain solution of specific concentration were applied topically before the 37° C. incubation. The control solution consisted of only Simms' Z TC-solution. The ouabain solutions

were made by dissolving the drug* in Simms' Z TC-solutions† to concentrations varying from 3×10^{-3} M to 3×10^{-8} M.

In another series of experiments ouabain was introduced into the anterior chamber after the cooling period. This was done by exchanging approximately 0.2 ml. aqueous for the same volume of ouabain solution through a needle introduced into the anterior chamber twice via the optic nerve.

Potassium effect. To study the effect of potassium on corneas exposed to ouabain, freshly enucleated eyes were cooled for 24 hours at 4° C. After this cooling period 10 drops of 3×10^{-4} M ouabain-Simms Z solution were applied to the cornea over a 2 hour period, during which time the eye was kept at +37° C. At this stage a balanced salt solution containing 60 mEq. K⁺ per kilogram H₂O was dropped on the cornea and the ouabain solution omitted. The corneal thickness was measured before the experiment, after the cooling period, and after 2 and 6 hours of incubation at +37° C.

Experiments in vivo. To study the effect of ouabain on the thickness of the rabbit cornea in vivo a 10^{-3} M ouabain-Simms' Z solution was dropped on the cornea over a 2 hour period. The thickness was determined before the experiment and 3 hours after the cessation of the dropping. The swelling was expressed in per cent of the normal corneal thickness.

The effect of potassium was also demonstrated in vivo. In four experiments a solution of 10^{-3} M ouabain in physiologic saline was dropped on the cornea during a period of approximately one hour. After an additional 2 hours the thickness which was measured before the experiment was again determined and 10 drops of a balanced salt solution containing 60 mEq. K⁺ per kilogram H₂O were applied to the cornea. The final thickness measurement was made after an additional 3 hours.

In all experiments utilizing potassium in vivo, the eyelids were closed by means of tape between the thickness determinations.

Results

Experiments in vitro. When refrigerated, the corneas showed an increase in thickness from a normal value of 0.35 to 0.42 mm. to 0.60 to 0.70 mm. When the eyes were subsequently incubated at +37° C., marked dehydration took place. This deturgescence was found to be significantly

*Ouabain USP, S. B. Penick and Company, New York, N.Y.

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larger when the control solution was topically applied than when it was introduced into the anterior chamber (Table I). The results are expressed as the deturgescence in per cent of the preceding swelling that took place during refrigeration.

Topical application of ouabain. Where the concentration of ouabain in the applied solution was increased from 3×10^{-6} M to 3×10^{-3} M, the deturgescence at 37° C. was reduced from 62 to 0 per cent of the preceding swelling. The deturgescence in the corresponding control experiments was 71 per cent (Table I). If this control value is regarded as the complete dehydration under these experimental conditions, a 50 per cent inhibition of deturgescence was found with ouabain concentrations between 10^{-4} M and 10^{-5} M (Table I).

Ouabain in the anterior chamber. For a ouabain concentration of 3×10^{-8} M (Table I), the dehydration was almost the same as that in the controls, i.e., 59 per cent. For an increasing concentration of ouabain to 3×10^{-5} M and higher, a complete inhibition of the deturgescence was noted. A 50 per cent inhibition of the effect in the controls was found for a ouabain concentration between 3×10^{-6} M and 3×10^{-7} M (Table I).

Without epithelium. If the epithelium was scraped off, the cornea was found to swell in the cold to the same extent as if it had been left intact. The deturgescence after 6 hours of subsequent incubation at +37° C. was, however, only two-thirds of that observed for intact cornea. If a $3 \times$

10^{-5} M ouabain solution was topically applied after the refrigeration, a complete inhibition of the deturgescence was noted. The same concentration had a greatly reduced effect on corneas with an intact epithelium.

The effect of high potassium concentration. When ouabain was applied to the cornea immediately after the cooling period, no deturgescence could be noted for 2 hours of subsequent warming. If, at this point, the ouabain solution was replaced by a balanced salt solution with a high potassium concentration, the corneal dehydration was 50 per cent of that of the control experiments for the same time. In the control experiments without ouabain the dehydration after 2 hours was 33 per cent of the preceding swelling.

Experiments in vivo. In ten experiments (Table II) 10 drops of 10^{-3} M ouabain in Simms' solution were applied to rabbit corneas in vivo over a period of 2 hours. After an additional 3 hours the thickness was found to have increased by 26 per cent \pm 4 (SD) over that measured before ouabain was applied. In 6 controls with Simms' solution alone, a slight decrease in thickness, -10 per cent \pm 2 (SD), was noted.

There was no sign of gross epithelial edema or fluorescein staining of the epithelium when the corneas were examined after the experiment with the slit-lamp biomicroscope.

In four experiments a swelling of 21 per cent during 2 hours was induced with

Table I. Effect of ouabain on corneal deturgescence in temperature reversal experiments

Ouabain concentration (M)	Topical application		Replacement of aqueous	
	No. of experiments	Dehydration in per cent of preceding swelling	No. of experiments	Dehydration in per cent of preceding swelling
0	18	71 \pm 3.1 (SEM)	11	59 \pm 5.1 (SEM)
3×10^{-8}			5	57 \pm 3.4
3×10^{-7}			7	39 \pm 2.6
3×10^{-6}	5	62 \pm 0.7	7	35 \pm 9.0
3×10^{-5}	5	55 \pm 8.1	9	5 \pm 3.3
3×10^{-4}	10	23 \pm 3.0	6	7 \pm 2.1
3×10^{-3}	7	0	5	0

Table II. Per cent increase in corneal thickness in vivo (rabbit) 5 hours after topical application of 10 drops 10^{-3} M ouabain

No. of experiments	Controls	No. of experiments	Ouabain
6	-10 ± 2 (SD)	10	$+26 \pm 4$ (SD)

ouabain topically. If the glycoside at this point was omitted and the cornea washed with a balanced salt solution containing K^+ in a concentration of 60 mg. per kilogram, the swelling was reversed and a thinning of 80 per cent of the preceding swelling after an additional 3 hours was demonstrated.

Discussion

The use of the temperature reversal technique has the advantage of giving rapid and clearly measurable changes in hydration. One can, therefore, with good accuracy measure the deturgescence. In the present series of experiments the cornea swelled in the cold from a thickness of approximately 0.40 mm. to about 0.65 mm. in 24 hours. The subsequent dehydration at $+37^\circ$ C. amounted to an average of 71 per cent of the swelling which resulted from refrigeration. These results are in reasonably good agreement with previously reported temperature reversal studies of the cornea.^{6, 8}

Since the eyes were kept in moist chambers and not immersed, the movement of water was limited to the endothelial side. When control experiments were carried out on eyes in which the aqueous was replaced by silicone oil, evaporation from the cornea or swelling due to uptake of water from the moist air did not amount to measurable quantities.

The experiments in vitro demonstrate clearly that the deturgescence which normally occurs at $+37^\circ$ C. could be greatly inhibited with ouabain. When it was applied topically, the inhibition was complete at a concentration of about 10^{-4} M. When

injected into the anterior chamber, complete inhibition was noted at about 3×10^{-5} M. Half maximal inhibition was noted under those two conditions at about 10^{-5} M and 3×10^{-6} M, respectively.

Half maximum inhibition of the Na-K activated ATPase in brain tissue, lens epithelium, and ciliary body was found with ouabain concentrations varying from 1.3×10^{-6} M to 7.8×10^{-8} M. These concentrations are slightly lower than those in the present study. However it must be emphasized that the concentrations of ouabain dropped on the cornea or injected into the anterior chamber do not necessarily represent the concentrations in contact with the endothelium. When ouabain is topically applied, it is likely that only a part of the glycoside reaches the endothelium, and this quantity is diluted by the interstitial fluids and the aqueous. When ouabain is injected into the anterior chamber, it is diluted by the remaining fluids of the eye. Consequently, the concentrations reported here have to be regarded as maximum concentrations. Furthermore, it is conceivable that the cells in a cell suspension are less viable and therefore more easily inhibited than the cells in the intact cellular membrane.

The experiments with K^+ in high concentration indicate not only an inhibition of the ouabain effect with this ion, but also that it is possible to reverse the ouabain effect.

For the in vivo experiments, several methods to apply the ouabain solutions were tried. Intravenous injection gave a too low concentration in the eye and, if increased amounts were given, the animal died. Injection into the anterior chamber and perfusion of the eye resulted in unpredictable swellings also of the control corneas, presumably due to an induced change in permeability in the endothelium. Topical application was therefore used. It was found that the corneas swelled substantially during 5 hours after the application of the glycoside. In the controls without ouabain a slight thinning was noted.

This thinning was considered to be due to evaporation from the cornea, secondary to a washing away of the oily layer of the precorneal tear film. It was possible also in vivo to demonstrate a reversal of the ouabain effect by increasing the potassium concentration.

A complete interpretation of the present results cannot be presented at this moment, and conclusions have to be based on analogies with other systems. An important question is that of the specificity of the ouabain effect. Does it have a specific effect on a cellular enzyme engaged in electrolyte transport, or does it have a nonspecific cytotoxic effect? The effective ouabain concentrations in this study are low enough for a specific effect in analogy with other tissues and, also, the reversibility of the ouabain effect with K^+ in high concentrations indicates a specific effect. Furthermore, a specific effect seems likely because of a recent study indicating that cardioinhibitory glycoside concentrations of less than $10^{-3}M$ are specific but higher concentrations are cytotoxic.²⁴

In other tissues ouabain has been shown to inhibit an electrolyte transport, and the present experiments would in analogy suggest a similar effect in the cornea. The deturgescence should then be brought about by a primary electrolyte transport across the endothelium and water should follow the electrolyte. To reach this conclusion with certainty, however, additional evidence is required from, for example, a demonstration of an inhibited electrolyte transport with isotopes. Finally, it is of great importance to also exclude an effect of ouabain on the permeability of the cellular barriers.

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