Effects of some metabolic inhibitors on the electrical potential difference and short-circuit current across the lens of the toad *Bufo marinus*

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The short-circuit current (SCC) across the isolated lens of the toad Bufo marinus *can be abolished by iodoacetate or ouabain but is not altered by 2,4-dinitrophenol, acetazolamide, or amiloride. Ethacrynic acid changes the SCC little and not in a manner comparable with that of ouabain. Ouabain and iodoacetate are effective only when placed at the anterior face of the lens from where they also reduce the potential difference (PD).*

Key words: toad lens, translenticular potential difference, short-circuit current, ionic permeability, iodoacetate, dinitrophenol, ouabain, ethacrynic acid.

The lens of the toad *Bufo marinus* exhibits a translenticular electrical potential difference (PD) and short-circuit current (SCC) in vitro.[1, 2] The anterior face of the lens has a PD of about 30 mv. positive with respect to the posterior side, while the SCC is equivalent to about 35 μA per square centimeter. An active transport of sodium occurs from the posterior to anterior side of the lens, and this can account for about 20 to 35 per cent of the SCC.

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Supported by The National Science Foundation Grant GB 12342 and National Institutes of Health Grants EY 00330 and EY 00160.

Manuscript submitted June 14, 1971; manuscript accepted July 23, 1971.

In the present experiments, the nature of these electrical parameters is further explored with the aid of various metabolic and enzyme inhibitors including: iodoacetate, 2,4-dinitrophenol, ouabain, ethacrynic acid, and acetazolamide.

Methods

Animals. Toads (*Bufo marinus*) weighing about 200 grams were obtained from a commercial supplier and kept on damp earth at 24° C.

Lens preparation. This was set up in a divided glass chamber so that the anterior and posterior sides were in apposition.[1, 2] The bathing solution had the following composition: (mM)Na^+, 104; K^+, 2.5; Ca^{++}, 1.0; Mg^{++}, 1.2 Cl^-, 74.5; HCO_3^-, 25; SO_4^{--}, 1.8; HPO_4^{--}, 2.9; gluconate^{--}, 1.0; glucose, 26. This was aerated and the pH was 8.7. Translenticular PD was measured, and current was passed through the lens by means of electrodes consisting of agar-Ringer–filled polyethylene tubing. The intralenticular electrode was a hand-pulled glass pipette with a tip diameter of 80 μ and a resistance of 150 KΩ or less. This electrode was filled with an agar-Ringer solution. The three

PD-measuring electrodes were connected to the recording equipment through calomel cells. Recording equipment consisted of a Keithley 200 B millivolmeter and a Heath EU-20 B recorder. SCC was delivered and measured with an automatic voltage clamp system.

Drugs. The ethacrynic acid, dihydroethacrynic acid, and amiloride were a gift from the Merck Institute for Therapeutic Research, West Point, Pennsylvania. Ouabain, 2.4-dinitrophenol (DNP), and iodoacetate (sodium salt) were from Sigma Chemical Company, St. Louis, Missouri, and acetazolamide was from Lederle Laboratories, Pearl River, New York.

Results

Effects of iodoacetate and 2,4-dinitrophenol on the electrical properties of the lens. In order to determine the coupling that exists between the translenticular SCC and metabolism, the effects of an inhibitor of anaerobic glycolysis, iodoacetate (IAA), and oxidative metabolism, 2,4-DNP, were examined.

Iodoacetate (2 mM.), when present in the solution bathing the anterior side of the lens, produced a prompt decline in the SCC (Fig. 1, Table I). The SCC was nearly zero after five to seven hours. In contrast, when the iodoacetate was placed in the solution bathing the posterior side of the lens, there was no significant change in the SCC as compared to control preparations. When DNP (10^{-4}M) was present in the fluids bathing both sides of the lens, there was no significant change in the SCC. Thus, it seems that the energy required for active translenticular transport of ions is derived from anaerobic glycolysis rather than oxidative metabolism. The iodoacetate can reach its site of action only from the anterior face of the lens, and, as this is where the epithelial layer of cells is also present, it probably reflects an action there.

The translenticular PD across the toad lens is the algebraic sum of the PD at the anterior and that across the posterior faces of the tissue. These were measured with the aid of a small pipette electrode inserted through the equator into the center of the lens. When iodoacetate (2 mM.) was present in the anterior solution, it produced a steady decline in the anterior PD. The PD at the opposite, posterior, face was slower to respond (Fig. 2) so that the magnitude of both PD's approached each other, and they were equal after about seven hours. The over-all translenticular PD was then zero. On some occasions, the PD at the anterior side declined further so that the translenticular PD was reversed by a few millivolts.

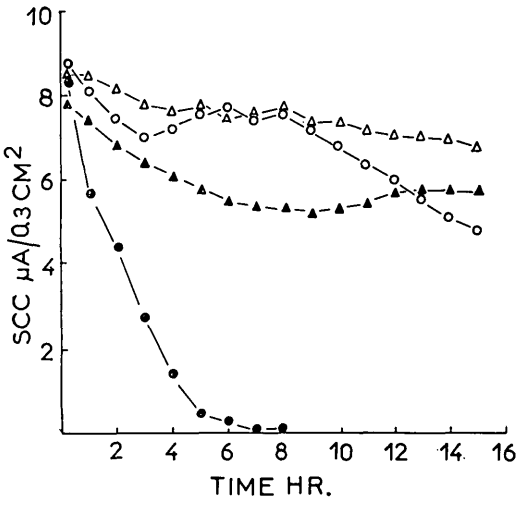

Fig. 1. Effects of 2 mM. of iodoacetate (anterior, ●—●, posterior O—O) and 10^{-4}M 2,4-dinitrophenol (both sides, △—△) on the SCC across the toad lens. Control, ▲—▲. Each point represents the mean of five observations. For a statistical analysis of the results see Table 1.

Effects of ouabain, ethacrynic acid, acetazolamide, and amiloride. The actions of a number of drugs known to inhibit processes connected with ion transport in ocular and other tissues were tested.

Na-K–activated ATPase is present in the epithelial tissue in the vertebrate lens.[3] This enzyme is closely linked to the process of active sodium transport and can be inhibited by ouabain[4] and, in some circumstances, in vitro by ethacrynic acid.[5] When ouabain (10^{-4}M) was present in the solutions bathing both sides of the toad lens, there was a slow decline in the SCC which approached zero levels after about 15 hours (Table I). A similar effect was also seen when it was present only in the

Table I. Effects of various drugs on the short-circuit current across the toad lens

Drug	*SCC as per cent of the initial value*				
	2 hr.	*4 hr.*	*6 hr.*	*8 hr.*	*12 hr.*
Control (5*)	87 ± 3.3	79 ± 5.1	73 ± 5.9	68 ± 5.5	72 ± 9.1
DNP 10^{-4}M both sides (5)	96 ± 2.3	89 ± 3.3	87 ± 5.8	89 ± 9.5	81 ± 9.4
IAA 2 mM. anterior side (5)	59 ± 7.1	15 ± 9.0	2 ± 10.7	−6 ± 9.1	
IAA 2 mM. posterior side (5)	86 ± 4.2	84 ± 10.0	88 ± 11.8	81 ± 9.1	64 ± 7.3
Control (6)	93 ± 6.4	81 ± 4.2	73 ± 4.5	68 ± 5.6	70 ± 12.9
Ouabain 10^{-4}M both sides (5)	76 ± 3.2	61 ± 2.4	47 ± 3.1	38 ± 3.0	21 ± 4.1
Ouabain 10^{-4}M anterior side (5)	70 ± 6.8	45 ± 8.0	36 ± 4.4	29 ± 7.0	9 ± 4.6
Ouabain 10^{-4}M posterior side (5)	89 ± 5.0	80 ± 7.4	71 ± 7.4	62 ± 9.4	44 ± 7.1
Control (5)	87 ± 3.3	79 ± 5.1	73 ± 5.9	68 ± 5.5	72 ± 9.1
Amiloride 10^{-4}M both sides (5)	89 ± 6.2	81 ± 7.2	64 ± 9.7	57 ± 10.8	57 ± 12.9
Control (5)	93 ± 4.7	82 ± 6.0	80 ± 12.0	84 ± 18.3	78 ± 15.3
Acetazolamide 10^{-3}M both sides (5)	100 ± 3.2	87 ± 4.7	77 ± 7.3	67 ± 6.2	59 ± 6.2
Control (5)	91 ± 2.8	86 ± 5.0	79 ± 7.1	74 ± 8.5	67 ± 8.7
Ethacrynic acid 10^{-4}M anterior side (7)	97 ± 3.3	90 ± 4.5	89 ± 7.9	95 ± 13.5	100 ± 13.9
Ethacrynic acid 10^{-4}M posterior side (7)	82 ± 2.6	69 ± 3.5	60 ± 4.6	56 ± 4.4	52 ± 4.4
Dihydroethacrynic acid 10^{-3}M posterior side (5)	87 ± 2.3	82 ± 4.5	77 ± 7.0	75 ± 8.0	68 ± 10.4
Dihydroethacrynic acid 10^{-3}M anterior side (5)	92 ± 3.5	86 ± 6.7	80 ± 9.7	73 ± 11.1	60 ± 9.1

Results as mean ± standard error.
*Number of experiments.

anterior bathing media, while there was little effect when it was present on the posterior side. Ethacrynic acid (10^{-4}M) did not have an effect comparable to that of ouabain. When present at either the anterior or posterior side of the lens, it did not produce a change in SCC which differed statistically from the control levels (Table I). However, the experiments with the ethacrynic acid on each side were carried out on paired lens preparations, and when these were compared the SCC was clearly less in the preparations with the drug present in the posterior than in anterior solution. This difference was not seen when an analogue diuretic lacking sulfhydryl-binding activity (dihydroethacrynic acid) was present. It is not clear whether the effect of the ethacrynic acid reflects a decreased SCC when it was present at the posterior side or an increased SCC when present at the anterior face, or both. Whatever the reason, the effect is not comparable to that of ouabain, so that it probably does not reflect an action on Na-K ATPase.

In a manner similar to that seen in the presence of iodoacetate, ouabain (10^{-4}M), when present at the anterior surface, reduced the PD across that side; the PD on the posterior side followed suit, and the translenticular PD became zero after about nine hours (Fig. 3).

The lens contains a high concentration of carbonic anhydrase,[6] but when an inhibitor of this enzyme, acetazolamide (10^{-3}M), was present in the media bathing both sides of the toad lens, there was no significant change in the SCC (Table I).

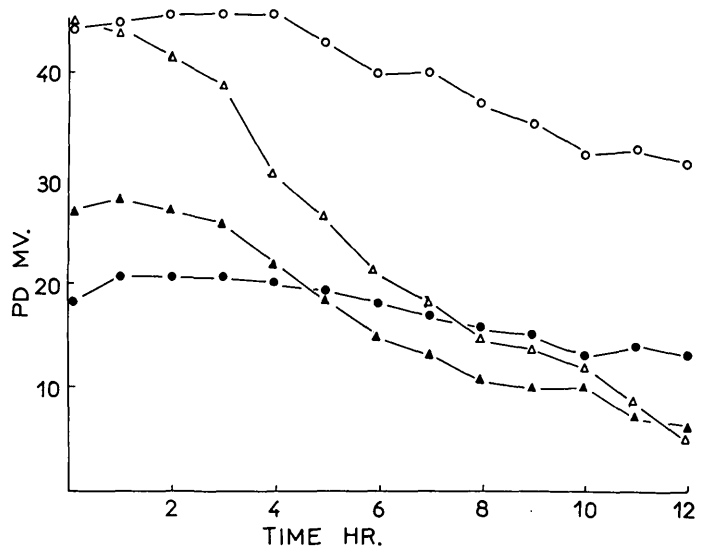

Fig. 2. Effects of iodoacetate (2 mM. at the anterior side of the lens) on the PD across the anterior (△—△) and posterior faces (▲—▲) of the toad lens. Control PD anterior face, ○—○; PD posterior face, ●—●.

Similarly, amiloride, which is a potent inhibitor of sodium transport in the kidney and across amphibian epithelial membranes,[7] also was without effect on the SCC.

Discussion

The SCC across the toad lens can be abolished by iodoacetate, but it is unaffected by dinitrophenol and is thus presumably dependent on anaerobic glycolytic processes. Ouabain also may abolish the SCC so that it seems the process is also dependent in some manner on Na-K ATPase and active sodium transport. It was interesting that both iodoacetate and ouabain were effective only when present at the anterior face of the lens, and this may reflect a site of action in or near the epithelial membrane present on that side. It has previously been shown[1, 2] that active sodium transport contributes only about 30 per cent of the translenticular SCC, so that if ouabain is acting only on this process it suggests that the remainder of the SCC is at least indirectly dependent for its integrity on the sodium transport. Such interactions are not uncommon or unexpected.

It seems to be of interest to compare the energy requirements for the observed SCC with the amount of energy which may be expected to arise from glycolysis. We have no information about glucose utilization in the toad lens, but in the rat lens at 20° C. it is 2.17×10^{-9} moles per hour for 100 mg. of tissue.[8] If the active ion transport is mediated by the breakdown of ATP to ADP, the energy available from this would be equivalent to 1.27×10^{-2} Joules per hour or 3.53×10^{-6} watts. The amount of electrical energy used in the present experiments by the toad lens is equivalent to 0.3

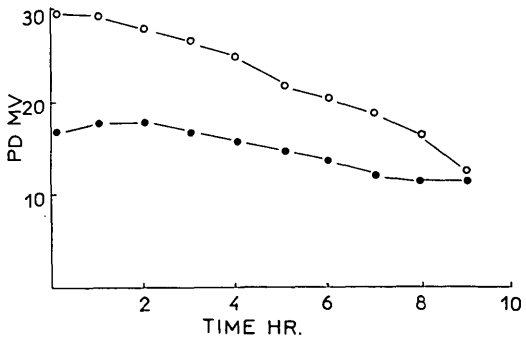

Fig. 3. Effects of ouabain (10^{-4}M at the anterior side) on the PD across the anterior (○—○) and posterior (●—●) faces of the toad lens. Each point represents the mean of 5 experiments.

$\times 10^{-6}$ watts [$(10\ \mu A)^2 \times 3{,}000\ \Omega$] or about eight per cent of the estimated available energy. This percentage will, of course, be affected by the fraction of the cells participating in the active transport but is of a magnitude which indicates that glycolysis could readily support the requirements for translenticular ionic transport across the lens.

The translenticular PD may primarily result from the metabolic activity at the anterior face of the lens; probably in the epithelial layer which is present there. While iodoacetate and ouabain reduce the SCC and PD across the anterior side of the lens when placed in contact with this, the posterior face is unresponsive to direct exposure to these agents. The PD across the posterior face of the lens, nevertheless, declines when these drugs are placed at the anterior side, but it seems likely that this reflects a dependence on the processes at that side. Iodoacetate, when added to the anterior side of the lens, produces a relatively quick decline in the PD across that side, while the PD at the posterior surface remained stable. After the PD across the anterior side declined by about 30 per cent, that across the posterior also started to decrease, and the PD across both surfaces then diminished to reach final levels of 0 to –7 mv. It seems possible that the PD across the anterior side is the result of both a diffusion potential and active ion transport, while that across the posterior is a diffusion potential only, the ionic gradients for which are created and maintained by the "Na pump" located at the epithelium. When the "pump" is inhibited, as by iodoacetate, these gradients cannot be maintained, and the PD across both surfaces declines.

These experiments provide additional evidence that the processes of ionic transport in the lens can be mainly supported by anaerobic metabolism and that the anterior epithelium of the lens is the site of the origin of electrical activity and ion transport. The translenticular SCC may provide a relatively simple and reliable parameter of the metabolic activity of the lens.

We are grateful to Miss Antonina Hochberg for her charming technical assistance.

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