Influence of calcium on retinal ATPases.

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The activity of Mg²⁺-ATPase and Na⁺-K⁺-ATPase was measured in media of calcium concentrations ranging from 10⁻⁸M to 10⁻⁷M, with retinal homogenates from rat and rabbit. In both species calcium stimulated the Mg²⁺-ATPase and inhibited Na⁺-K⁺-ATPase. In the rat, activity of Na⁺-K⁺-ATPase fell by 75% as calcium was increased from 10⁻⁸M to 10⁻⁷M and reached 90% inhibition only at 10⁻⁵M. By contrast, the activity in the rabbit fell gradually to a 90% inhibited state, over the range 10⁻⁷M to 10⁻⁵M. Calcium activated the Mg²⁺-ATPase by a maximum of 50% in each species, at a concentration of 10⁻³M in the rat and over a broader concentration range between 10⁻⁷M and 10⁻⁵M in the rabbit. It is postulated that maintenance of intracellular calcium at low levels by the Ca²⁺-activated Mg²⁺-ATPase or other cellular mechanisms is essential for the activity of the membrane-bound Na⁺-K⁺-ATPase of the retina.

Previous work in our laboratories has demonstrated that the activity of the Na⁺-K⁺-stimulated ATPase in homogenates of whole retina and pigment epithelium-choroid is inhibited almost completely by 2 mM calcium. In these studies the enzymatic activity was measured only at very low (<10⁻⁷M) or very high (10⁻³M) concentrations of calcium. It can be argued that the normal intracellular concentration of free ionized calcium may not bear any relation to the millimolar levels found to inhibit Na⁺-K⁺-ATPase activity in the homogenates. Yet, it is the intracellular level of calcium which influences the activity of this enzyme in vivo. For this reason, experiments using Ca²⁺-ethylene glycol tetraacetate (EGTA) buffer mixtures have been undertaken with rat and rabbit retinas to measure enzymatic activity over a wide range of calcium concentrations in order to assess more accurately the relationship between calcium and the physiological activity of the Na⁺-K⁺-ATPase. Concomitantly, we have observed a Ca²⁺-activated Mg²⁺-ATPase which is capable of playing a role in the regulation of intracellular calcium.

Methods. Rats, weighing 150 to 250 gm, were anesthetized with chloroform, and one retina, isolated as previously described, was homogenized in 3.5 ml of H₂O. Retinas of approximately 2 kg were killed by intravenous injection of air, and the isolated retina was homogenized in 10 ml of H₂O. Use of water, which caused osmotic lysis of cells and cell organelles, yielded a homogenate as active as a lyophilized preparation and avoided the need for detergents such as Triton X-100 which was found to inhibit the Na⁺-K⁺-ATPase. ATPase activity of 0.1 ml aliquots was measured in a final volume of 0.5 ml containing 2 mM ATP, 2 mM MgSO₄, 5 mM KCl, 130 mM NaCl, 30 mM Tris HCl, and varying concentrations of calcium and EGTA. Free calcium was calculated according to the method of Schatzmann. Ouabain, when present, was 0.1 mM. The pH of all solutions at 37°C was 7.4, and they were all approximately 305 milliosmolar. ATPase activity was measured by determining the phosphate liberated from ATP as described previously. Na⁺-K⁺-ATPase is the difference between the activities recorded in the presence and absence of ouabain. This difference was equivalent to the difference in activities recorded in the presence of calcium and in the absence of calcium.

Fig. 1. Effects of varying the concentration of calcium on the relative magnitude of Na⁺-K⁺-ATPase activity in homogenates of rat retinas (closed circles) and rabbit retinas (open circles). For the rat, 100% activity is 5.5 µmol Pi per milligram of protein per hour, and each point represents the mean ± 1 S.E. (n = 4). For the rabbit, 100% activity is 2.5 µmol Pi per milligram of protein per hour, and each point is the average of three homogenates.
corded in calcium-free media in the absence and presence of sodium ion, as shown previously. Ca²⁺-activated Mg²⁺-ATPase was measured in the presence of ouabain and is the difference in activities in media with 2 mM EGTA and media with free calcium concentrations regulated by Ca²⁺-EGTA buffers. Absence of sodium from these media caused a small uniform lowering of ATPase activity at all Ca²⁺ concentrations from 10⁻⁹ M to 10⁻⁴ M. Hydrolysis of ATP was linear with time up to 60 min and at protein concentrations from 25 to 80 μg per cuvette, so long as not more than 20% of the ATP was broken down. Protein was determined by the method of Lowry et al.

Results and discussion. Fig. 1 shows the effects of different concentrations of ionized calcium on the activity of the Na⁺-K⁺-stimulated ATPases of rat and rabbit, expressed as percentage changes. The maximal Na⁺-K⁺-ATPase value, taken as 100%, was recorded in both species at the lowest calcium concentration, calculated to be approximately 10⁻⁸ M. It is clear that calcium has little effect on the enzyme activities when its concentration is less than 10⁻⁸ M. However, when the concentration is increased toward 10⁻⁴ M, enzyme activity in the rat homogenate is sharply depressed to 25% of the maximum, a level which is maintained as the calcium concentration is increased to 10⁻⁴ M. In the rabbit homogenate, inhibition of the Na⁺-K⁺-ATPase is more gradual, activity declining in an almost linear fashion from 90% at 10⁻⁴ M calcium to 35% at 10⁻⁴ M. Finally, at 10⁻⁴ M calcium the enzyme activity in both species is inhibited by more than 90%.

These results clearly demonstrate that calcium inhibits the Na⁺-K⁺-stimulated ATPase at concentrations which are considerably below the millimolar quantities used in previous studies. The enzyme activity of the rat appears to be particularly sensitive to changes in its concentration less than 10⁻⁷ M. However, when the concentration is increased toward 10⁻⁴ M, enzyme activity in the rat homogenate is sharply depressed to 25% of the maximum, a level which is maintained as the calcium concentration is increased to 10⁻⁴ M. In the rabbit homogenate, inhibition of the Na⁺-K⁺-ATPase is more gradual, activity declining in an almost linear fashion from 90% at 10⁻⁴ M calcium to 35% at 10⁻⁴ M. Finally, at 10⁻⁴ M calcium the enzyme activity in both species is inhibited by more than 90%.

These results clearly demonstrate that calcium inhibits the Na⁺-K⁺-stimulated ATPase at concentrations which are considerably below the millimolar quantities used in previous studies. The enzyme activity of the rat appears to be particularly sensitive to changes in its concentration between 10⁻⁷ M and 10⁻⁴ M. Nevertheless, it is equally clear that if the intracellular level is maintained below 10⁻⁷ M, full activity of the Na⁺-K⁺-ATPase can be expressed. It appears therefore that the expression of the inhibitory action of calcium on this enzymatic activity and hence its physiological significance in the retina depend very much upon the mechanisms which control the intracellular calcium concentration, particularly at those sites where the Na⁺-K⁺-ATPase is located.

One mechanism by which the cytosolic concentration of calcium may be regulated is the Ca²⁺-activated Mg²⁺-ATPase of mitochondria. Fig. 2 shows that calcium ions, in a narrow concentration range around 10⁻⁴ M, increase the Mg²⁺-ATPase activity of the rat retina by 50%. Between 10⁻⁷ M and 10⁻⁴ M, activity is partially inhibited as a result of competition between Mg²⁺ and Ca²⁺ for the ATP. In the rabbit, it can be seen that calcium again exerts its effect over a broader range than in the rat and that the maximum stimulation is between 10⁻⁵ M and 10⁻⁴ M, a concentration range some 100- to 1000-fold higher. When one compares Fig. 1 with Fig. 2, it can be seen that the free calcium concentration in the cytosol which produces a maximum stimulation of the Ca²⁺-activated Mg²⁺-ATPase is of the same order of magnitude as that which produces a 50% inhibition of the Na⁺-K⁺-ATPase.

The work of Berman et al. using a heavy fraction of cattle rods, suggests that a Ca²⁺-activated Mg²⁺-ATPase with the characteristics described in this report is located in the mitochondrial fraction of the inner segment of the photoreceptor cell. The plasma membrane of the inner segment contains the predominant fraction of the ouabain-sensitive Na⁺-K⁺-ATPase of the retina, the activity of which we show to be strongly dependent upon the calcium concentration. If the mitochondrial Ca²⁺-activated Mg²⁺-ATPase is associated with sequestration of this ion, then this retinal enzyme might well be expected to regulate the cytosolic calcium concentration and, in turn, the active transport of...
sodium and potassium ions. Although we have no evidence to date whether this particular system operates in an intact retina, nevertheless the results of the present study show that there must be some mechanism to hold the intracellular concentration of free calcium at a low level in order to prevent inhibition of the Na\(^+\)-K\(^+\)-ATPase.

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REFERENCES


The effect of ascorbic acid on experimental acid burns of the rabbit cornea. Patricia Wishard and Christopher A. Paterson.

The corneas of albino rabbits were subjected to 45 sec, 12 mm, 2.3N hydrochloric acid burns. Of 18 eyes in nine rabbits receiving no treatment (controls), 11 (61%) developed ulceration sometimes progressing to descemetoceles and perforation. Of 17 eyes in nine rabbits receiving a daily subcutaneous injection of ascorbic acid (0.5 gm/kg), only one eye (5.9%) developed an anterior stromal ulcer. The difference in incidence of ulceration between the control and ascorbate-treated eyes was statistically significant (p < 0.01). The aqueous humor level of ascorbate in untreated animals was 6.0 ± 0.6 mg/dl compared to 33.0 ± 2.7 in the treated group. This study therefore demonstrates that subcutaneous administration of ascorbic acid significantly raises the aqueous humor level of ascorbic acid in severely acid-burned eyes, thereby largely preventing the characteristic development of corneal ulceration. The mechanism of this effect is presumably the same as previously described for alkali-burned eyes.

Both alkali and severe acid burns of the cornea are known to result in epithelial defects and corneal ulceration and perforation. It has been demonstrated that following alkali burns of the rabbit cornea, the level of ascorbic acid in the aqueous humor is greatly reduced as a consequence of damage to the ciliary processes, which are normally responsible for the active transfer of ascorbic acid from the blood into the aqueous humor. Thus the cornea becomes deficient in ascorbic acid, which is essential for collagen synthesis. Treatment of alkali-burned eyes with subcutaneous ascorbic acid or topical ascorbic acid significantly reduces the incidence of corneal ulceration and perforation.

These results have been interpreted to suggest that the ulcerating cornea, following an alkali burn represents not only a tissue undergoing breakdown of collagen by collagenases but also a tissue unable to exercise its normal reparative process because of a lack of ascorbic acid. Elevation of aqueous humor levels of ascorbic acid by exogenously administered ascorbic acid supports the synthesis of collagen, thereby permitting more rapid corneal healing in the face of collagen breakdown and ulceration.

Corneal changes following acid burns of the eye have been less well investigated. They are generally considered less damaging because acids do not penetrate the globe as rapidly as do alkalis. However, after severe ocular acid burns, the resultant corneal changes follow a course similar to that after alkali burns. It has also been shown that the aqueous humor levels of ascorbic acid are reduced for several days following acid burns. This finding has been confirmed and extended in our laboratory (unpublished observation).

The present paper describes the influence of subcutaneously administered ascorbic acid on the corneal changes of rabbits subjected to severe corneal hydrochloric acid burns.