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Sub Title	
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Publisher	共立薬科大学
Publication year	1983
Jtitle	共立薬科大学研究年報 (The annual report of the Kyoritsu College of Pharmacy). No.28 (1983.) ,p.90- 91
JaLC DOI	
Abstract	
Notes	抄録
Genre	Technical Report
URL	https://koara.lib.keio.ac.jp/xoonips/modules/xoonips/detail.php?koara_id=AN00062898-00000028-0090

Sodium-Potassium ATPase in Normal and Cataractous Human Lenses*

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In the present work, the activity of Na/K ATPase in different regions of normal and various types of cataractous human lenses has been determined by a sensitive radioactive method. It is shown that with increasing severity of opacity the activity of this enzyme decreases, the loss in actitity being apparent first in the inner regions of the lens.

Normal human lenses were obtained from the New York Eye Bank for Sight Restoration Inc. within 24 hours of death and assayed immediately. The cataractous lenses were assayed within one hour of extraction. After removal of the capsule and the epithelium, the lens was placed in 1 ml of 0.05 M imidazole, 1 mM DTT and 0.1 mM EDTA buffer pH 7.5, and shaken gently for 30' at 0° to remove the conrtical region. The residual lens nucleus was removed carefully and suspended in another test tube in the same buffer. The cortical suspension and the residual lens were each homogenized with all glass Potter homogenizer by hand at 0°. Capsule-epithelium was homogenized in 1 ml of the buffer using a polytron Homogenizer and a PT7 probe at 0°. Aliquots of the homogenate were incubated in two tubes containing 120 mM NaCl, 20 mM KCl, 10 mM $MgCl_2$, 20 mM imidazole buffer pH 7.5 and 5 mM ATP (5×10^4 cpm/μmole). Ouabain (1 mM) was added to the duplicate tube to determine the amounts of ouabain sensitive ATPase. The enzyme reaction was terminated by addition of 50% TCA. After centrifugation, the ATPase was measured by assaying the released inorganic phosphate by two methods : the colorimetric method or the isotopic method. The only modification made to the published isotopic method was the addition of 50% TCA in place of 6% SDS for termination of the enzyme reactions, since addition of SDS to lens homogenate does not completely stop the ATP hydrolysis.

Using the isotopic method for the determination of released inorganic phosphate, the total ATPase and Na/K ATPase activities in the capsule-epithelium, cortex and nucleus of 14 normal human lenses have been measured. Results with the ouabain sensitive Na/K ATPase indicate that in the human lenses, the enzyme is not solely distributed in the epithelial cells, but is also present in the cortical and nuclear regions. The total Na/K ATPase values have a slight decrease in activity of the enzyme with aging similar to the nuclear region and statistical analyses indicate a slope of -1.55×10^{-3} with an axtrapolated 0 age of 0.49. The slope is statistically significant with $p=0.037$. Thus it would appear form this analysis that with aging there is a gradual loss of activity of

* 本報告は *Current Eye Res*, 2, 327—334 (1982/1983) に発表

Na/K ATPase in the inner regions of the lens. A similar situation is found in the total Na/K ATPase.

To detect and localize the changes in the Na/K ATPase activity in cataract, the lenses were classified according to CCRG procedure and placed into two groups. Lenses classified as CX_{1-2}, N_{1-2}, NS_{Y-B}were in Group 1 and CX_{3-4}, N_{3-4}, NS_{Y-B} in Group 11. The values of the Na/K ATPase activities in the different section of these lenses were appreciably lower in the total lens and in the cortical and nuclear regions, when compared to the normal lens. In the epithelial region, only in the Group 11 lenses was there a significant lowering in the Na/K ATPase activity.

To compare the mean values of the enzyme activity, normal lenses younger than 45 years of age were excluded from the caluculation since all the cataractous lenses are older than 45 years. Thus the age distribution of normal and cataractous lenses are similar, with a mean age of 67 years for Group 11 cataract. The mean values of the anzyme activity for normal and cataractous lenses further corroborates our findings that in severe cataract (Group 11), the enzyme activity is minimal in all regions of the lens, whereas in slight to moderate cataracts (Group 1), although a significant decrease in the enzyme activity is observed in the cortical and nucleus sections, no significant decrease is observed in the epithelial layer ($P=0.428$). Thus the lowering of the Na/K ATPase activity in cataract is unrelated to age and is associated with the lens pathology.