ACTA OPHTHALMOLOGICA VOL. 56 1978

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THE EFFECT OF X-IRRADIATION ON THE SODIUM-POTASSIUM-ACTIVATED ADENOSINE TRIPHOSPHATASE (Na-K-ATPase) ACTIVITY IN THE EPITHELIUM OF THE RAT LENS

A Histochemical and Biochemical Study

BY

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The epithelial Na-K-ATPase activity of the rat lens was studied after X-irradiation at intervals of three to ninety days. The enzyme was demonstrated histochemically by light microscopy and it was measured biochemically by a fluorometric method. Neither histochemical nor biochemical changes of Na-K-ATPase content of the lens epithelium were observed during the development of cataract.

In whole-mount preparations the enzyme activity was localized in the cell membranes. However, one month after radiation a few peripheral cells had in addition a precipitate over the whole cell.

The unaltered Na-K-ATPase content in the epithelium suggests that the hydration of the lens after X-irradiation is primarily caused by changes in the passive permeability properties of the cell membranes and not by a decreased capacity of the active cation pump.

Key words: radiation cataract – rats – Na-K-ATPase – lens epithelium.

Biochemical and morphological studies have shown that diffuse swelling of the lens is an early phenomenon in the development of many types of experimental cataract (Lambert & Kinoshita 1967; Michon & Kinoshita 1968a,b; Kuwabara et al. 1969; Kuck 1970; Iwata & Kinoshita 1971; Harris & Gruber 1972; Palva & Palkama 1978). The accumulation of water into the lens can occur by a variety

Received February 18, 1978.

of mechanisms. Normally the sodium ion concentration is maintained at a low level in the lens due to limited permeability and an active extrusion mechanism (cation pump). An increase in the permeability of cellular membranes to this ion may lead to a net influx of the ion into the lens. At the initial stage this may be compensated for by an increased efflux of potassium ions, but when sodium gain exceeds potassium loss, water begins to accumulate into the lens (Duncan & Croghan 1969). The same mechanism applies to the impaired function of the cation pump system. Swelling of the lens may also be due to an excessive accumulation of osmotically active substances into the lens, as suggested by Kinoshita et al. (1962, 1968) in sugar cataracts.

The cation pump appears to be located in the lens epithelium (Kinsey & Reddy 1965). The system actively extrudes sodium ions from the lens in exchange for potassium, thus regulating the normal volume of the lens. The sodium-potassium activated ATPase (Na-K-ATPase), which is found in high concentrations in the epithelium of the lens (Bonting et al. 1963; Palva & Palkama 1974, 1976; Neville 1977) has been assumed to be an integral part of this active cation pump mechanism.

In an earlier study from our laboratory it has been shown that the ultrastructure of the lens epithelium is markedly affected by X-irradiation (Palva & Palkama 1978). The aim of the present study was to investigate the possible role of the epithelial Na-K-ATPase on the development of X-ray induced cataract. The activity of Na-K-ATPase in the lens epithelium was demonstrated by both histochemical and biochemical methods after X-irradiation of the rat lens *in vivo*.

Material and Methods

Seventy-five female Sprague-Dawley strain rats were used. The animals were four months old and their weight varied between 200 and 300 g. The left eye of each rat was exposed to an air dose of 1500 r of X-rays from a 200 kV source through 0.5 mm copper. The other radiation factors were 10 ma, 86 r per min and a 40 cm target lens distance. The irradiation technique was the same as that employed in our previous work (Palva & Palkama 1978). Light anaesthesia with sodium pentobarbital preceded the irradiation. The right eye was protected against radiation by a 5 mm thick lead sheet.

Histochemical studies

At intervals of 3, 7, 30, 60 and 90 days after the X-ray treatment, the animals (three in each group) were quickly killed and both eyes were then treated

identically to demonstrate the ATPase activity. In the incubation solution ATP was used as substrate, lead as precipitating agent, and magnesium, sodium and potassium as activators (Palva & Palkama 1976).

Biochemical studies

At the same intervals as in the histochemical procedure the animals were killed for biochemical analysis of Na-K-ATPase activity in the lens epithelium. Isolated epithelium and capsule preparations were frozen in liquid nitrogen. Each group contained 10–15 control (right eye) and irradiated (left eye) preparations. Five preparations were pooled together in order to have sufficient tissue for analysis and homogenized at 0°C in glass grinders containing 0.3 M mannitol for 1.5 min (Riley 1964). The ATPase activity was determined fluorometrically using the method described by Härkönen et al. (1972). The Na-K-ATPase activity was estimated as the difference between total ATPase and Mg-ATPase activity. The protein concentration was measured by the method of Lowry et al. (1951).

Results

In whole-mount preparations of control animals the Na-K-ATPase activity was localized only in the membranes of the polygonal epithelial cells (Fig. 1).

Three or seven days after the irradiation no changes were observed either in the intensity or localization of the reaction product or in the form of the epithelial cells.

Thirty days after irradiation the centrally located cells showed variance in their size and shape. The cellular membranes, however, seemed to be intact and the ATPase activity displayed normal intensity (Fig. 2). In some peripherally located cells at the equatorial region of the lens the reaction product was also present inside the cell (Fig. 3).

Sixty days after irradiation no alteration in the amount of reaction precipitate in the cell membranes was visible (Fig. 4) but the number of cells showing a reaction in the cytoplasm was increased in the peripheral epithelium (Fig. 5). Almost all epithelial cells had an irregular configuration at this stage (Fig. 4).

Ninety days after irradiation, when the first biomicroscopically demonstrable opacities became visible in the posterior subcapsular area of the lens, the dark-brown precipitate still showed normal intensity in the cell membranes (Fig. 6). The deformative changes in the shape and size of the epithelial cells were further increased and the cell membranes were often broken. The nuclei, which

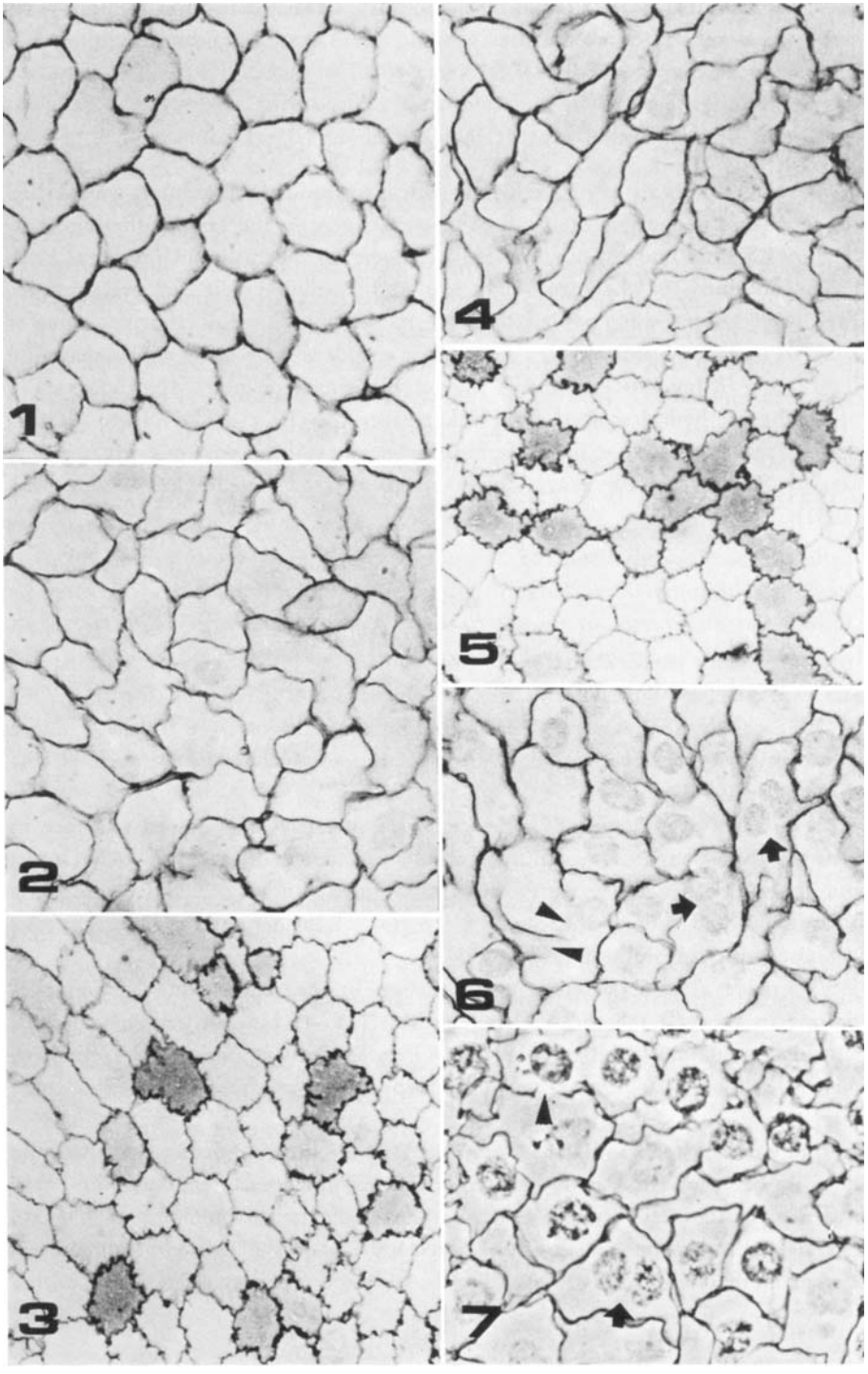
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Table I.
Na-K-ATPase activity in isolated epithelium and capsule preparations of control (normal) and X-irradiated rat lenses from three to 90 days after irradiation.

Preparations	Na-K-ATPase activity* μmole × mg^{-1} prot × min^{-1}	Range μmole × mg^{-1} prot × min^{-1}
Control	$2.68 \pm 0.16 \times 10^{-2}$	$2.35 - 3.07 \times 10^{-2}$
X-irradiated	$2.75 \pm 0.10 \times 10^{-2}$	$2.53 - 2.99 \times 10^{-2}$

* Mean values ± SE, where each value is the mean of 2–3 determinations using 5 lenses per determination. The difference from the control is not significant, $P > 0.35$.

Figs. 1–7.

1. A whole-mount preparation of a control lens epithelium demonstrating ATPase activity. The reaction product is restricted to the cell membranes. × 500.

2. A whole-mount preparation of central epithelium thirty days after X-irradiation. The ATPase activity displays normal intensity. The size and shape of cells show greater variance than in the control preparation. × 500.

3. A whole-mount preparation of peripheral epithelium thirty days after X-irradiation. The ATPase activity is at the cell membranes and some cells show a diffuse precipitate over the cytoplasm. × 500.

4. A whole-mount preparation of central epithelium sixty days after X-irradiation. No alteration of ATPase activity can be seen when compared with Fig. 1. The shape of the cells is quite irregular. × 500.

5. A whole-mount preparation of peripheral epithelium sixty days after X-irradiation. The relative number of cells showing cytoplasmic staining for ATPase is increased. × 500.

6. A whole-mount preparation of central epithelium ninety days after X-irradiation. The ATPase activity is limited to the cell membranes. At some sites the cell membranes have broken (arrow heads). Binuclear cells are also visible (arrows). × 500.

7. A phase contrast micrograph of the same preparation as in Fig. 6. The nuclei contain coarse and clumped chromatin. A binuclear cell is visible (arrow) and the cell membrane is disrupted (arrow head). × 500.

were nonreactive, refracted light in the peripheral epithelium, and this made them visible without staining (Fig. 6). In phase contrast microscopy, rather coarse, clumped chromatin was seen in such nuclei (Fig. 7). Some cells contained two nuclei (Figs. 6 and 7).

No loss of biochemically measured Na-K-ATPase activity was observed in X-irradiated lenses, as compared with the controls (Table I).

Discussion

The X-irradiated lens is known to swell during the development of the cataract. Ultrastructural studies have revealed both hydropic epithelial cells and fibres with vacuole formation already one week after X-ray exposure (Palva & Palkama 1978). Lambert & Kinoshita (1967) showed that the cation transport remains normal in spite of water accumulation into the X-irradiated lens. They suggested that the characteristic posterior subcapsular opacities observed biomicroscopically are due to the fact that the anterior epithelium alone possesses the capacity to correct the ionic and water disturbances resulting from altered membrane permeability.

Hydration of the lens may also be initiated by accumulation of osmotically active metabolities into the lens, as was suggested in sugar cataracts (Kinoshita et al. 1962, 1968). Galactose feeding is followed by marked swelling of the rat lens fibres as shown electron microscopically by Kuwabara et al. (1969). Duncan & Croghan (1969) have, however, cast doubt on such a polyol accumulation theory and suggested that the opacification, not only in sugar, but also in other forms of cataract, is due either to a decreased cation pumping capacity or to an increase in the passive sodium permeability of the cellular membranes.

Iwata & Kinoshita (1971) provided evidence that a depressed Na-K-ATPase level probably accounts for the initiation of recessively autosomally transmitted cataract in mice. Decreased Na-K-ATPase activity has also been reported by Gupta & Harley (1975) in human senile cataractous lenses and by Fournier & Patterson (1971) in sugar cataract of rat, but not until the manifestation of mature cataract. On the other hand, Michon & Kinoshita (1968b) reported a normal cation transport and a marked increase in the permeability of cellular membranes in experimental miotic cataract. Similar results were obtained in X-ray exposed lenses of rabbits by Lambert & Kinoshita (1967) and in X-irradiated human erythrocytes by Sheppard & Beyl (1951). Nordmann & Mandel (1955) found an increased ATPase activity in the cortex of X-irradiated rabbit lenses during the first days following radiation, whereas later the differences were no longer consistent.

No significant changes were observed in the biochemically measured Na-K-ATPase activity of the lens epithelium and the histochemically demonstrable activity localized, as in controls, in the epithelial cell membranes. However, one month after the irradiation some equatorial cells showed diffuse cytoplasmic staining, which may reflect injury of the cell membrane. The shape and size of the epithelial cells became more irregular during the experiment and multinuclear cells were often seen. These changes are probably due to degeneration and swelling of individual cells.

The histochemical and biochemical results presented in this paper suggest that the Na-K-ATPase activity of the epithelial cell membranes of the lens is not primarily affected by X-irradiation. On the other hand, it must be noted that X-irradiation causes relatively early swelling of the epithelial cells (Palva & Palkama 1978). Thus, it can be suggested that these early changes in the lens after X-irradiation are in the first place due to an increased passive permeability of the cell membranes to cations and not to a defect in the active cation pumping system.

Acknowledgment

The author wishes to express his gratitude to Mrs. Hilkka Sulkava for skilled technical assistance.

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