The movement of procion dye in the crystalline lens

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Fluorescent procion dyes when iontophoresed into lens fibers through a glass capillary microelectrode appear, on gross observation, to be within a single fiber only. Fluorescent microscopy reveals, however, that the dye diffuses appreciably from one cell to another. The movement is primarily along a single column of fibers at low currents, but spreads to adjacent columns at higher currents. The dye does not enter the fibers from the extracellular space. Fibers adjacent to the electrode track seem able to exclude the dye. It is concluded that movement of dye from one fiber to another must occur across low resistance "tight junctions."

Key words: procion dye, tight junctions, diffusion, iontophoresis, microelectrode, lens fibers, cell coupling.

Procion dyes have been used in the neurosciences for determination of neuronal geometry.1, 2 These dyes, particularly procion yellow (M4RS), diffuse readily in cytoplasm, do not bind appreciably to protein, are retained in the cell, and show appreciable fluorescence. Since the dye molecule is negatively charged, it can be injected by iontophoresis through micropipettes into single cells where it will diffuse for considerable distances. Procion yellow has also been found to readily cross electrotonic junctions between adjacent cells.3 An electrotonic junction is an area of low electrical resistance between two adjacent cells. Current passed through a microelectrode placed in one of the cells will induce a voltage change not only in that same cell but will also cross the junction and induce a voltage change in the second cell as well. This junction represents a functional continuity between adjacent cells.

"Tight junctions" which might be electrotonic junctions have been reported in the lens by a number of investigators.4, 5 Use of procion dyes in the lens has also been previously described.6 The purpose of this investigation was to determine if procion dyes iontophoresed through micropipettes into lens fibers would remain exclusively in those fibers or would diffuse from fiber to fiber. Such diffusion would be anticipated if true electrotonic junctions existed between adjacent lens fibers.

Method

Common frogs, Rana pipiens (two to three inches in length) were decapitated. The eyes were enucleated and the posterior globe dissected away. The globe with lens still suspended from
Fig. 1. Typical dye ribbon seen in intact lens through dissecting microscope (x33).

Fig. 2. Fluorescent photomicrograph of low current (1 to 2.5 nA.) dye infusion pattern in lens (x330).

its zonules was pinned in place in a Plexiglas chamber with a transparent Sylgard (Dow Corning, Midland, Mich.) layer in the bottom. The globe was then immersed in Ringer's solution and placed in the microelectrode apparatus. The eye was illuminated from below through the cornea and pupil by use of a high-intensity fiber-optic illuminator. The preparation was observed through a high quality dissecting microscope at 25 to 50x magnification.

Microelectrodes were filled with 6 per cent procion scarlet (H-RNS) or a 50-50 mixture of 6 per cent procion scarlet—6 per cent procion yellow.9 The electrodes had tip diameters of 0.6 to 0.8 microns and resistances of about 20 megohms. All penetrations were made through the posterior lens surface by use of a David Kopf stepping motor-driven hydraulic micromanipulator. The stepping motor was pulsed by a logic circuit device designed by the author.10 This device allowed penetration in one micron steps at rates of from 0.5 to 100 microns per second. The total number of steps could also be preset so as to drive the electrode tip a specified distance into the lens. The surface of the lens was precisely identified (within 1 μ) by a tone change in a voltage-controlled audio monitor produced when the electrode touched the lens. Depths in the lens were within 10 μ of those desired for depths greater than 100 μ (verified by histology). Using this device, repeated penetration with small-tipped electrodes was possible without the tips breaking.

When the tips were in place, dye was iontophoresed into the lens through the use of a logic circuit pulse generator designed by the author.11 Trains of 5 negative pulses to 1 positive pulse were used to keep an electrode at constant impedance by preventing clumping of procion in the tip. Pulses caused 1 to 50 nA square pulses of 500 msec duration to pass through the electrode. Voltage at the microelectrode and current were constantly measured using WPI M4 electrometers and a Tektronix 565 oscilloscope.

Dye was later identified either by dissecting microscope observation of the intact lens at 50x or through fluorescent microscopy using a Zeiss ultrashot microscope. Lenses were prepared for fluorescent microscopy by fixing for 12 hours in neutral formalin and then dehydrating and paraffin embedding by standard techniques. Sections were made of 6 μ thickness and were observed unstained and unoverslipped under the fluorescent microscope.

Results

Dye injected into the lens at low current (1 to 2.5 nA.) appears to stay within a single fiber when the intact lens is viewed at 50x. Fig. 1 shows a typical dye ribbon. Passage of such current for three to four hours results in a ribbon which runs from suture to equator. No evidence of lateral spread of dye is seen unless currents of 25 to 50 nA. are used.9

Fluorescent microscopic examination of histologic sections shows a quite different picture. Sections were cut in a plane perpendicular to the dye ribbon and perpendicular to the plane of the lens equator. Sections included here were taken from in front of or behind the actual penetration site so that the electrode track is not seen. Fig. 2 shows such a fluorescent section. It can be seen that background fluorescence is essentially nonexistent. The area of intense fluorescence (arrow) in-
Movement of procion dye in crystalline lens

The movement of procion dye in crystalline lens indicates the level of penetration of the electrode tip. It can be seen that dye does not remain localized at the level of the tip. Rather it moves toward the lens surface and toward the nucleus by as much as 50 to 75 cell thicknesses in each direction. This movement takes place almost exclusively in a single column of fibers. Occasionally, dye can be seen in two to three lens fibers in adjacent columns (double arrows). These fibers are intermittently spaced and are only found in columns immediately adjacent to the main one.

Currents of 25 to 50 nA produce a different pattern (Fig. 3). Appreciable lateral spread of dye into four to five fiber columns on each side of the main column can be seen. The dye still appears to move much more in a horizontal direction than in a lateral direction. The dye does not appear to be in the extracellular space since a dark line (arrow) can be seen separating adjacent fiber columns which contain dye. The intermittent light-dark areas along a single column are small groups of fibers containing different concentrations of dye.

Dye does not enter lens fibers from the bath for 0 to 1 per cent bath concentrations (made isosmotic by addition of Na-reduced Ringer’s solution). It enters the capsule readily but does not penetrate the lens epithelium, fibers, or extracellular space. At higher bath concentrations the dye appears toxic to the epithelium which disintegrates. The dye can then be found in the dead surface cells and throughout the extracellular space, but not in the fibers (Fig. 4).

Insertion of a 5 μ tip diameter dye-filled electrode deep into the cortex with subsequent application of two to four atmospheres of pressure results in the rapid “filling” of one of the cortical zones of discontinuity. Dye then diffuses entirely throughout the extracellular space without entering fibers. The pattern seen is identical to Fig. 4.

Cells immediately adjacent to a large dye-filled electrode track do not take up dye indiscriminately (Fig. 5). They appear to have intact membranes. They do not appear damaged.

Discussion

The observations reported here might be explained by assuming that the electrode damages a number of fibers in a particular column as it penetrates. When dye is ejected it simply diffuses up the electrode track, enters the damaged fibers, and diffuses along them. This explanation is unlikely since dye is also found in fibers 50 to 75 μ deeper than the electrode tip. Damage of these fibers is not anticipated and no electrode track exists to them. Also,
Fig. 5. Fluorescent photomicrograph showing fibers immediately around abnormally large electrode track (×330). Areas where less dye has entered adjacent fiber columns (arrow) can easily be seen.

Fig. 5 shows that even fibers immediately adjacent to the electrode track do not all take up dye. They do not, as a group, appear to be appreciably damaged.

The dye patterns observed could not be explained by diffusion into fibers from the extracellular space since the data indicates that the dye does not penetrate fiber-surface membranes.

The most reasonable explanation for the data is that dye, when placed in a lens fiber, does not remain solely in that fiber. Rather, it diffuses from fiber to fiber. Since the dye did not enter fibers from the extracellular space and since no evidence for diffusion through damaged fiber membranes was found, this diffusion must have occurred directly through specialized cell to cell junctions. Diffusion of molecules as large as these dyes has been reported only across electrotonic-type junctions. Presumably then, these junctions must exist in the lens. The dye movement is greatest in the horizontal direction. This is to be anticipated since the hexagonal lens fibers have more total area adjacent to fibers above and below than they do to fibers to the side. A different permeability of junctions at different places in the fiber cannot be ruled out.

The greater lateral spread at higher currents is expected. Higher currents result in more dye ejected per unit time. This results in a greater diffusion gradient in all directions.

The study suggests that in the crystalline lens, adjacent fibers are not functionally independent of one another. Rather, their cytoplasm is “joined” through low-resistance junctions. These junctions allow the passage of even relatively large substances from one cell to another. This arrangement would facilitate the movement of metabolites and ions throughout the lens. Electrical equilibration should also result.

REFERENCES