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Cytochalasin D Disrupts Outer Segment Disc Morphogenesis In Situ in Rabbit Retina

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Exposure of rabbit retina to cytochalasin D (CD) via a sin- gle intraocular injection results in basal rod outer segment (ROS) and cone OS (COS) discs with abnormally large diameters; the overgrown OS membranes extend along ei- ther the cell outer or inner segment. Twenty-four hours after the injection, basal ROS and COS discs appear to have recovered their normal diameter, indicating the revers- ibility of this drug's effect. These data support both the evagination hypothesis for disc morphogenesis and the hy- pothesis that f-actin's role at the ROS base is to regulate the initiation of membrane evagination and disc diameter. Invest Ophthalmol Vis Sci 30:339-342, 1989

The presence of actin at the base of the rod outer segment has been established in a variety of verte-[2]

brate species, using antibodies to actin[1]. and the f- the retina was unknown, but considerably less than actin-specific probe, fluorescent phalloidin,[3] leading to the suggestion[2] that this contractile protein might perform some major role in disc morphogenesis. New disc membrane is added to the ROS base by evagina- tion of the ciliary membrane.[4] Such membrane movements recalllamellipodial extensions ("ruflles") of motile cells, including growing neurons, which can be attributed to f-actin[5] and may involve a treadmill- ing mechanism.[6]

F-actin's role in membrane movements was estab- lished in part with studies employing a family of drugs, the cytochalasins. These compounds bind to the barbed ends of actin microfilaments, inhibiting both association and dissociation of g-actin sub- units.[7,8] The most potent cytochalasin is cytochalasin D (CD), which has the added advantage of not affect- ing the cell glucose transporter, as do some of the other cytochalasins.[9] While there is still uncertainty as to CO's actual in vivo effects,[10] it is generally ac- cepted that CD inhibits local rearrangements of f- actin that rely on actin polymerization, particularly in cells undergoing shape changes.[11,12]

Recently, CD was shown to deregulate ROS disc morphogenesis in *Xenopus* eyecups maintained in vitro.[13] This deregulation was manifested in basal OS discs of abnormally large diameter. We report here that CD administered to the rabbit retina by intraoc- ular injection disrupts OS disc morphogenesis in an identical fashion; moreover, disc diameter appears to recover approximately 24 hr after the drug is injected.

Materials and Methods. Rabbits were pigmented adults with apparently normal eyes when examined with an indirect ophthalmoscope. They were kept under a 12:12 light/dark regime and the eyes were injected at 3 hr after light onset. One eye of each animal was the experimental eye; the other served as a control. Experimental eyes were injected with 100 1 of a stock mixture consisting of 5 1 CD (Calbio- chem, San Diego, CA), dissolved to 5 mg/ml in DMSO, plus 995 1 of sterile balanced salt solution (BSS; Alcon, Fort Worth, TX). This stock could be stored frozen, but was resterilized by filtration prior to use. Final concentrations of the injected fluids were thus 25 g/ml CD and 0.5% DMSO in sterile BSS. Final concentration in the extracellular space of that of the injections themselves.

Rabbits were deeply anaesthetized with an intra- muscular injection of Rompun and Ketamine prior to intraocular injections. With the anaesthetized rab- bit lying on its side, the sclera of the uppermost eye was gently grasped with a forceps to steady it. A sterile 30-gauge needle was used to penetrate the cornea at its periphery, with care taken to avoid the iris, and then withdrawn. Gentle pressure was applied to the sclera near the corneal incision, draining off some intraocular fluid in order to compensate for the in- traocular pressure added by the subsequent injection. The site of injection was through the temporolateral sclera; the needle was inserted approximately 3.5 mm into the posterior vitreous cavity, taking care not to hit the lens. Any sensation of back-pressure during the slow injection was compensated for by another release of fluid from the corneal incision. Following injection, both eyes were given drops of Lidocaine at approximately hourly intervals for 4 hr as the animal recovered from anaesthesia. None of the animals showed any signs of discomfort, nor did any corneal, scleral, or intraocular pathology develop following the procedures. Rompun and Ketamine anaesthesia was repeated prior to euthanasia, which was

accom- plished with Nembutal administered by intracardiac puncture. All procedures were performed in accordance with the ARVO Resolution on the Use of Ani- mals in Research.

One animal was killed at each time point (8 or 24

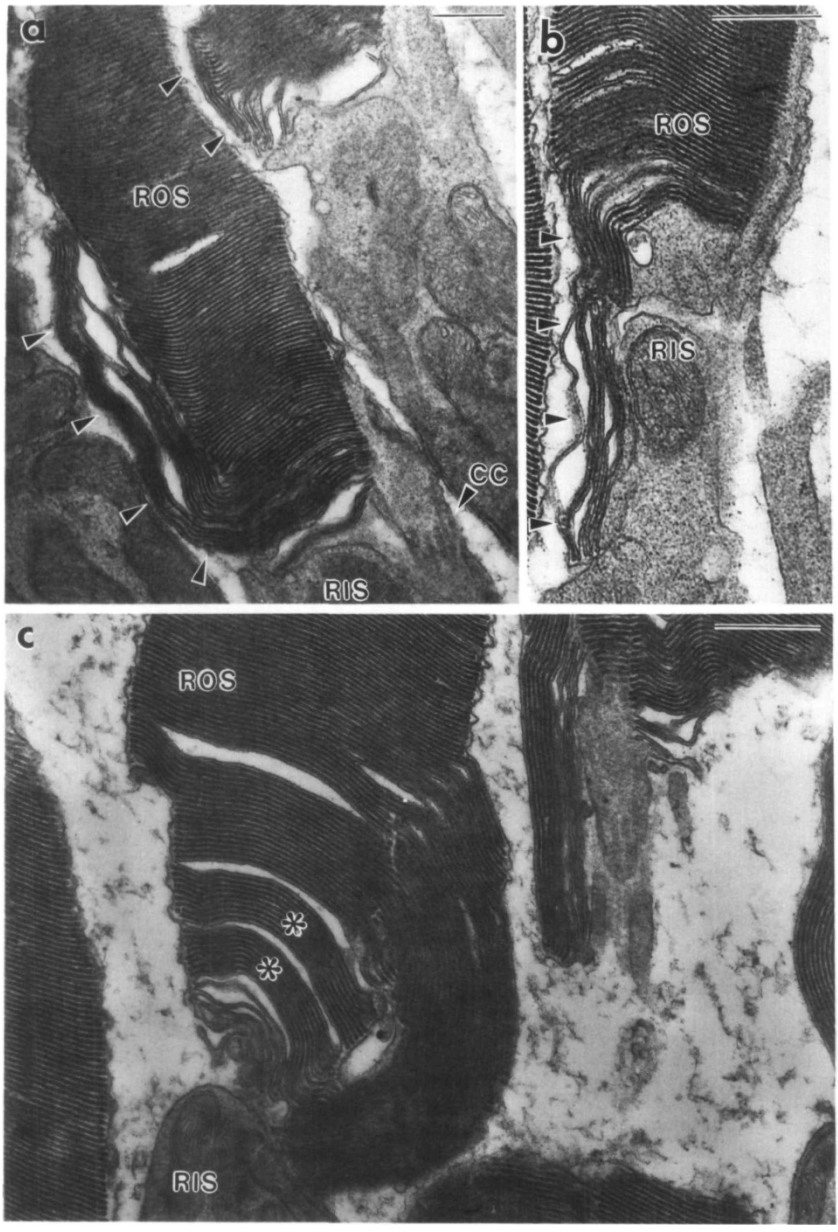

Fig. 1. Electron micrographs of rabbit rod photoreceptors, showing the abnormally large diameter (and resulting abnormal displacement) of basal ROS membrane discs after CD injection. ROS = rod outer segment; RIS = rod inner segment; CC = connecting cilium. Scale bars = 5 *m*. **(a)** From a retina fixed 8 hr after CD injection. Overgrown basal ROS discs (arrows) extend upward (sclerad) along the ROS plasma membrane. At upper right, a second ROS with overgrown basal discs is seen, but with overgrown discs extending downward (vitread) instead. **(b)** From a retina fixed 8 hr after CD injection. Overgrown ROS discs extend downward (vitread) along the RIS. (c) From a retina fixed 24 hr after CD injection. Overgrown ROS discs extend downward in two cells. In the cell at left, however, the most basal discs appear to be of a more normal diameter(*), suggesting that after 24 hr the drug's effect on regulation of disc assembly has been reversed.

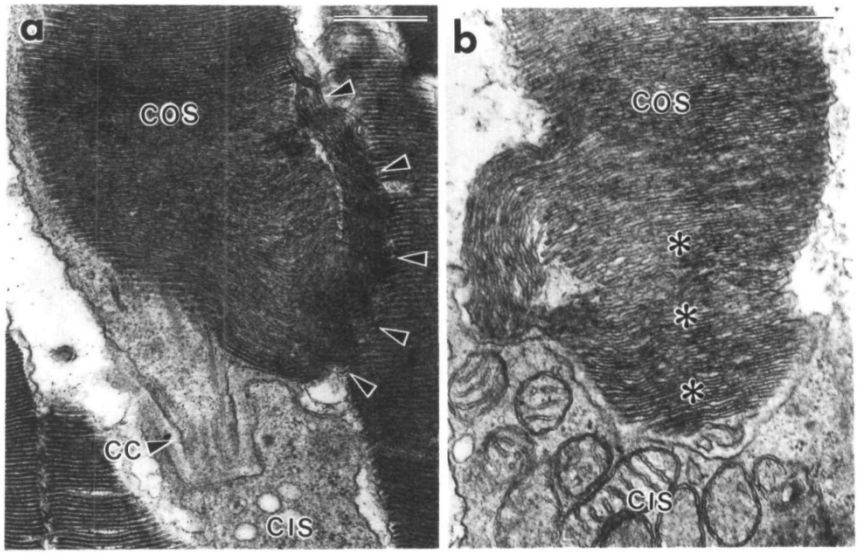

Fig. 2. Electron micrographs of rabbit cone photoreceptors, showing the abnormally large diameter (and resulting abnormal displacement) of basal COS membrane discs after CD injection. COS =cone outer segment; CIS =cone inner segment; CC = connecting cilium. Scale bars = 5 I'm. (a) From a retina fixed 8 hr after CD injection. Overgrown basal COS discs (arrows) extend upward (sclerad) along the COS. (b) From a retina fixed 24 hr after CD injection. Overgrown COS discs extend downward (vitread) to the CIS. As in the ROSs at 24 hr after injection, the most b sal discs appear to have a more normal diameter(*), suggesting that the drug's effect on regulation of disc assembly has been reversed.

hr post-injection) and the eyes processed for conven- tional transmission electron microscopy. Eyecups were fixed overnight in 1% paraformaldehyde + 1% glutaraldehyde in phosphate buffer (pH 7.2). After rinsing in buffer, the eyecups were cut into quadrants and postfixed for 1.5 hr with 2% osmium tetraoxide in phosphate buffer. Following ethanol dehydration and infiltration with propylene oxide, the quadrants were embedded in Araldite and thin-sectioned on an ultramicrotome (LKB, Bromma, Sweden). Gold or silver sections were collected onto copper mesh grids, stained with uranyl acetate and lead citrate, and ex- amined with a Philips 300 electron microscope (Eindhoven, The Netherlands).

Results. In retinas fixed 8 hr after CD injection, most photoreceptor OSs had basal discs with abnor- mally large diameters, so that the nascent discs appeared to "spill over" the side of the cell. In both rods and cones from CD-treated retinas, overgrown basal OS membranes were observed to extend sclerad along the OS (Figs. 1a, 2a) or vitread along the IS (Figs. 1b, c, 2b). In either case, the overgrown membranes maintained contact with the cell from which they grew. This is in marked contrast to the normal situa- tion in which new discs achieve the diameter of their older, mature counterparts and, following closure, are displaced further up (sclerad) the ROS. Over- grown discs were not.observed in retinas from the control (opposite) eye of any animal.

In retinas fixed 24 hr after CD injection, overgrown discs were still observed in many ROSs and COSs; but the most basal discs had an appearance suggestive of recovery of their normal diameter (Figs. lc, 2b). The overgrown OS membranes remained apposed to inner or outer segment.

Discussion. Williams et al [13] have previously shown that isolated photoreceptors from *Xenopus* eyecups maintained in CO-supplemented culture medium have f-actin domains that collapse into focal accu- mulations in the cell body, a typical effect of this drug.[12] The loss of the f-actin domain at the photore- ceptor OS base clearly established the sensitivity of that domain to CD. The same study revealed one consequence of that sensitivity, namely that *Xenopus* ROS and COS disc morphogenesis in the presence of CD was abnormal: the basal discs were overgrown, having a larger-than-normal diameter. Concurrent radiolabelling showed that the abnormal discs were assembled during drug exposure, rather than deriving from preexisting discs which were simply altered structurally by the drug. That report[13] thus consti- tutes the first experimental evidence that actin local-

ized at the OS base is important for disc morphogen- esis, and further, provides experimental evidence for the evagination hypothesis of disc morphogenesis proposed by Steinberg and his colleagues.[4]

The present study provides additional evidence for

the evagination hypothesis[4] from an in situ applica- tion of the drug and extends the observations on CD's disruptive effect on disc morphogenesis to a mam- malian species. Furthermore, the morphology ofboth ROSs and COSs from rabbit retina fixed 24 hr after
CD injection suggests a recovery of normal disc di- ameter, ie, a reversal of the drug's effect on the f-actin domain at the OS base, probably due to washout from the vitreous of what was essentially a pulse of CD (cf. ref. 14). In contrast, the report by Williams et al[13] was based on continuous delivery of the drug in vitro and correspondingly they did not record any evidence for recovery. We therefore conclude that it is likely that regulation of OS disc morphogenesis by f-actin occurs similarly in both mammals and am- phibians and is not an artifact of the in vitro system.

Key words: actin, cytochalasin D, disc morphogenesis, photoreceptors, retina

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