If we assume, that $g_{Na}$ is constant at various $[Na]_i$ then

$$I_{Na_1}/I_{Na_2}=(V_m-E_{Na_1})/(V_m-E_{Na_2}),$$

where $I_{Na_1}$, $I_{Na_2}$ are maximal values of $I_{Na}$ and $E_{Na_1}$, $E_{Na_2}$ are the reversal potential at various $[Na]_i$. Reversal potential for the sodium ions was calculated from Nernst's equation:

$$E_{Na}=RT/F \cdot \ln [Na]_o/[Na]_i.$$

For these conditions the experimental value of $I_{Na_1}/I_{Na_2}$ was found to be 1.21 and its calculated value obtained by using the Nernst's equations to determine $(V_m-E_{Na_1})/(V_m-E_{Na_2})$ was equal to 1.32. Therefore, we assume that this satisfactory agreement between the experimental data and the theoretical predictions points to an adequate intracellular perfusion.

Cardiac cells isolated enzymically from adult rat hearts are morphologically intact[6,10] and maintain electrical activity. The electrical response of the isolated cells appears to be similar to that observed in intact tissue; action potentials recorded from these cells exhibit fast upstrokes and low plateaus[11]. The fast upstroke is probably due to the fast inward TTX sensitive current identified in the present paper.

The results obtained in this study show the possibility of applying a voltage-clamp method to single cardiac cells. Combination of a voltage-clamp method with the intracellular dialysis technique may have advantages in studies of the dependence of the membrane ionic currents upon the intracellular medium.

1 We wish to thank Dr N. Veselovsky of Kiev Bogomoletz Institute of Physiology for methodical consultations and Dr R. Gilmour of Krannert Institute of Cardiology, Indianapolis, Indiana, USA, reviewing the manuscript.

2 E.A. Johnson and M. Lieberman, A. Rev. Physiol. *33*, 479 (1971).
3 G.W. Beeler and J.A.S. McGuigan, Prog. Biophys. molec. Biol. *34*, 219 (1978).
4 T.J. Colatsky and R.W. Tsien, Nature *278*, 265 (1979).
5 P.G. Kostiuk, O.A. Krishtal, V.I. Pidoplichko and N.S. Veselovsky, Neuroscience *3*, 327 (1978).
6 J. Rajs, M. Sundberg, G.V. Sundby, N. Danell, G. Tornling, P. Biberfeld and S. Jakobson, Exp. Cell Res. *115*, 183 (1978).
7 K.G. Lee, T.A. Weeks, R.L. Kao, N. Akaike and H.M. Brown, Nature *278*, 269 (1979).
8 L. Belardinelli, R. Rubio and R.M. Berne, Pflügers Arch. *380*, 19 (1979).
9 A.L. Hodgkin and A.F. Huxley, J. Physiol. (Lond.) *117*, 500 (1952).
10 T. Powell, E.M. Steen, V.W. Twist and N. Woolf, J. molec. cell. Cardiol. *10*, 287 (1978).
11 T. Powell, D.A. Terrar and V.W. Twist, J. Physiol. (Lond.) *284*, 148B (1978).

## Cone myoid elongation and rod myoid contraction are inhibited by colchicine in the trout retina

M. Anctil, M.A. Ali and P. Couillard[1]

*Department of Biology, University of Montreal, Montreal (Canada H3C 3J7), 6 August 1979*

*Summary.* Retinal photoreceptors of lower vertebrates undergo photomechanical changes (elongation or shortening) in response to light or dark. Colchicine, a microtubule-disrupting drug, blocks cone, but not rod elongation. Instead, rod shortening is blocked by this drug, thus suggesting that different mechanisms mediating these responses are involved in rods and cones.

A remarkable adaptive feature of eyes in lower vertebrates is the translocation of photoreceptor (rod and cone) inner and outer segments and retinal epithelium pigment granules, seemingly to control the amount of light to which the photosensitive elements are exposed under various photic conditions. These movements are collectively called 'retinomotor responses' and have been extensively studied in the past 60 years [2-4]. The movements of rods and cones occur in opposite directions and appear to be mediated by contraction and elongation of the myoid, a specialized region of the photoreceptors located between the ellipsoid and the perikaryon.

The exact nature of the subcellular or supramolecular mechanisms underlying these movements is still poorly understood[4]. Couillard[5] and Burnside[6] proposed models based on studies of motility in unicellular organisms. In these proposals, a cooperative interplay of microfilament-mediated contraction and microtubule-mediated elongation accounts for these responses in photoreceptor myoids. Substantial evidence in favour of this model came from studies in which the presence of actin-like and myosin-like filaments, as well as microtubules, were reported in cone myoids and ellipsoids[6,7]. Moreover, it was shown that cytochalasin B prevents cone contraction during the dark-to-light transition, whereas colchicine disrupts microtubules and prevents cone elongation during the reverse transition in the retina of a fish capable of these responses[6,8,9].

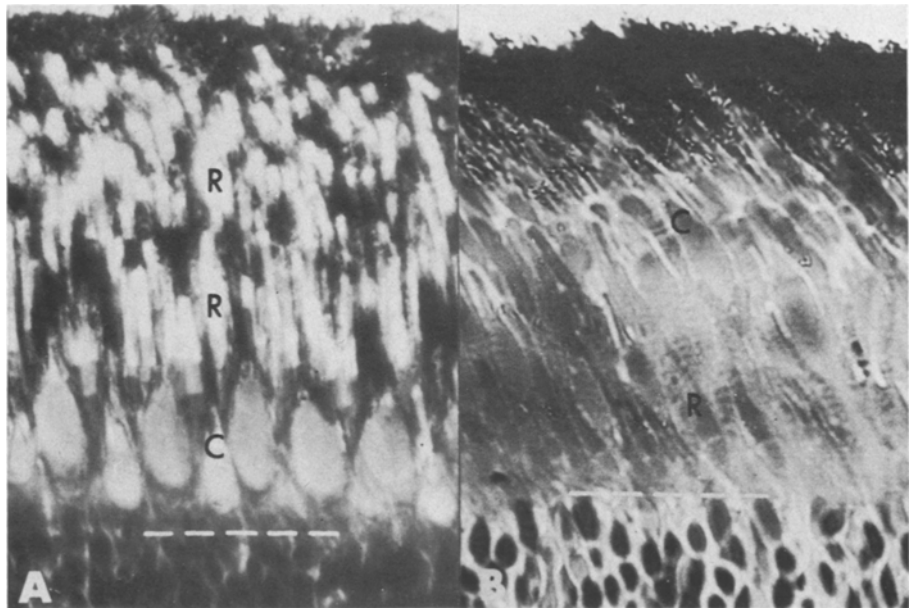
The latter experiments allowed observations on cones only. Therefore, it remains crucial to know whether rod translocations are mediated by mechanisms similar to those postulated for cones. We report here that the retinomotor behaviour of rods in the trout retina is affected by colchicine in a manner opposite to that described in cones, thus suggesting that different mechanisms may be involved.

Young specimens of brook trout, *Salvelinus fontinalis* (14–15 cm in length), were kept for 1 week in a recirculating water tank at 8–10 °C prior to experiments, with a 12-h light period. The irradiance at the water surface was about 17.5 mW/cm$^2$. The right eye of each fish was injected intraocularly with 50 µl of 1 mM colchicine (Sigma) dis-

Rod and cone positions in the photoreceptor layer of light- and dark-adapted trout retina

| | Dark → light | | Light → dark | |
|---|---|---|---|---|
| | Cones | Rods | Cones | Rods |
| Control | 0.30 ± 0.02 | 0.39 ± 0.06 | 0.56 ± 0.06 | 0.14 ± 0.04 |
| Colchicine | 0.29 ± 0.03 | 0.36 ± 0.05 | 0.27 ± 0.03 | 0.32 ± 0.03 |

Measurements of the distance between the external limiting membrane (ELM) and Bruch's membrane were made in 2 fish per experiment. The positions of double cone outer segment tips and rod ellipsoid tips within this space were expressed as ratios of the total distance. Owing to the dispersion of rods in 2 superimposed layers, only those within the vitread - most 30 µm from the ELM were measured. Each value represents the mean (± SD) of 10 measurements performed in transverse sections of ventral retina, at distances of every other 10 µm within a space of 200 µm.




Photomicrographs of transverse sections of the retina in a trout *(Salvelinus fontinalis).* A, light-adapted right eye treated with colchicine, then put in the dark. Rods (R) and cones (C) remained in the light-adapted positions. B, light-adapted left eye (control) injected with saline, then put in the dark. Rods (R) and cones (C) contracted and elongated respectively. ×590. - - - -, location of the external limiting membrane used for measurements in the table.

solved in freshwater teleost Ringer, and 50 µl of Ringer was injected in the left eye (control). The final concentration of the drug in the vitreous chamber was estimated to be about 0.1 mM. In order to obtain complete adaptation, the animals were first exposed to dark or light for at least 1 h prior to injection. They were then lightly anesthetized with MS 222, injected, and kept in the same photic condition for another 1–1.5 h to allow for diffusion and equilibration of the drug in the vitreous chamber and retina. Following this, the photic conditions were reversed for 1.25–1.5 h and the animals were sacrificed. The eyes were rapidly excised, and the retinas were fixed in a 1% glutaraldehyde-paraformaldehyde mixture in a 0.12 M phosphate buffer. After postfixation in 1% osmium tetroxide in the same buffer, the retinas were dehydrated and embedded in Araldite. Sections of 1–2 µm were prepared and examined with a Zeiss photomicroscope. Control and experimental transverse sections were compared from corresponding ventral and dorsal retinal areas, roughly equidistant between the fundus and ora serrata.

In dark-adapted trout injected with colchicine prior to light adaptation, rods elongated and cones contracted in a manner comparable to uninjected controls. Thus colchicine, in particular, had no effect on rod elongation. On the other hand, if light-adapted retinas are treated with colchicine and then induced to dark-adapt, both rod contraction and cone elongation are blocked by the drug (figure, table). As a whole, these results show that colchicine, in the trout retina, has no effect on light-induced retinomotor responses whereas it blocks dark-induced responses in both types of visual cells. In the case of cones, the inhibition of myoid elongation by colchicine was quite expected and adds to the evidence in favour of the involvement of a microtubular sliding mechanism[8,9]. Much more puzzling is the fact that colchicine, far from inhibiting rod elongation, instead blocks rod contraction (table).

The possibility that rods were prevented from contracting because of obstruction by cones in the vitread position (contracted state), rather than by colchicine, was next examined. For this purpose, we used a teleost fish, *Stizostedion vitreum,* whose retinomotor movements involve only rods, cones remaining immobile in the vitread position[10,11]. When eyes of *Stizostedion* were treated with colchicine, rods were prevented from contracting during the light-to-dark transition, but elongated normally during the reverse adaptation. Thus blockade of rod contraction appears to be mediated by colchicine directly. Interestingly, light micrographs of colchicine-treated, dark-adapted grunt *(Haemulon)* retinas published by Warren and Burnside[9] show evidence of blockade of rod myoid contraction, at least in the vitread-most 20–30 µm of the photoreceptor layer.

Colchicine is known to disrupt labile microtubules in many cell systems[12], and there has been one report of the disappearance of cone myoid microtubules after exposure to a concentration of colchicine similar to ours[9]. Assuming that colchicine interfered selectively with a microtubular system in our experiments, it is difficult to explain how microtubules could mediate elongation in cones and contraction in rods. In recent ultrastructural studies, microtubules, but not microfilaments, were present in trout rod myoids, whereas both structures were found in trout cones[13]. Moreover, microtubules are apparently not involved in rod elongation, as suggested by our negative results of colchicine treatment on light adaptation. The thrust of all these observations supports our proposal that rod movements in teleost retinomotor adaptations are subserved by mechanisms which differ significantly from those operating in cones.

1 Acknowledgments. This work was supported by a grant from the Quebec Ministry of Education (FCAC). We thank Carole Marullo for technical assistance.
2 L.B. Arey, J. comp. Neurol. *26,* 121 (1916).
3 M.A. Ali, Vision Res. *11,* 1225 (1971).
4 M.A. Ali, in: Vision in Fishes, p.313. Ed. M.A. Ali. Plenum Press, New York 1975.
5 P. Couillard, in: Vision in Fishes, p.357. Ed. M.A. Ali. Plenum Press, New York 1975.
6 B. Burnside, J. supramolec. Struct. *5,* 257 (1976).
7 M. Anctil and M.A. Ali, Rev. Can. Biol. *36,* 113 (1977).
8 B. Burnside, J. Cell Biol. *78,* 227 (1978).
9 R.H. Warren and B. Burnside, J. Cell Biol. *78,* 247 (1978).
10 E.S. Zyznar and M.A. Ali, Can. J. Zool. *53,* 180 (1975).
11 M.A. Ali and M. Anctil, J. Fish. Res. Bd Can. *34,* 1467 (1977).
12 J.B. Olmsted and G.G. Borisy, A. Rev. Biochem. *42,* 507 (1973).
13 E. Ferrero, M. Anctil and M.A. Ali, Rev. Can. Biol. *38,* p.249 (1980).