

**EFFECTS OF GENISTEIN, A TYROSINE KINASE  
INHIBITOR, ON LIGHT ADAPTIVE CELLULAR  
AND SYNAPTIC PLASTICITY IN THE OUTER  
RETINA OF A TELEOST FISH (CARP)**

by

**A. H. SUBRATTY<sup>1\*</sup>, S. N. HAAMED<sup>2</sup> and M. B. A. DJAMGOZ<sup>2</sup>.**

*<sup>1</sup>Department of Health and Medical Sciences, Faculty of Science, University of Mauritius, Réduit, Mauritius and <sup>2</sup>Neurobiology Group, Department of Biology, Imperial College of Science, Technology & Medicine, Sir Alexander Fleming Building, London SW7 2AZ, UK.*

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**ABSTRACT**

The effects of genistein, a protein tyrosine kinase inhibitor, on light adaptation-induced morphological (cellular and synaptic) plasticity were studied in the retina-eyecups of carp (*Cyprinus carpio*). In particular, the effects of genistein on the photomechanical movements of cones and the spinules of horizontal cells were quantitatively evaluated. The data suggested that genistein significantly blocked both light adaptive processes. It is concluded, therefore, that light adaptation of the teleost retina could involve activation of tyrosine kinases(s). This conclusion agrees with previous findings that multiple neuromodulators and protein kinases control retinal light adaptation.

**Keywords :** Genistein, tyrosine kinase, light adaptation, plasticity, cone, photomechanical, movement, horizontal cell, spinules, retina, carp.

The retina of teleost fish is remarkably plastic; responding to dark @ light adaptation with a variety of morphological and corresponding physiological changes (Djamgoz *et al.* 1990, 1995a, 1996, 1999). The most common examples of such plasticity occurring in the outer retina are the photomechanical movements (PMMs) of photoreceptors (Ali, 1975; Burnside & Nagle, 1983), and the spinules of horizontal cells (HCs) (Wagner, 1980; Wagner & Djamgoz, 1993). Thus, during dark @ light adaptation, cones contract towards the external limiting membrane whilst HCs extend finger-like extensions called “spinules”. The neurochemical control of these processes is not yet fully understood. Dopamine is thought to be a major light adaptive modulator (Djamgoz & Wagner, 1992) and, indeed, is released in the teleost retina during light adaptation (Kirsch & Wagner, 1989). However, some dopamine-independent effects have also been described (Baldrige & Ball, 1991; Douglas *et al.* 1992; Djamgoz *et al.* 1996). More recently, nitric oxide (NO) has been suggested to be a novel, light adaptive neuromodulator (Greenstreet & Djamgoz, 1994; Djamgoz *et al.* 1999; Sekaran *et al.* 1999).

As regards the signal transduction pathways associated with the light adaptive neuromodulatory control mechanisms, some work has been done on the possible role of second messengers and associated protein kinases (PKs). Direct evidence has been presented for several different serine/threonine PK activities being involved in photoreceptor PMMs (Pagh-Roehl *et al.* 1996). In the case of spinules, activation of D1 type dopamine receptors and accumulation of cAMP in HCs are consistent with involvement of PKA (Van Buskirk & Dowling, 1981; Kirsch *et al.* 1991). In fact, Lasater (1987) has shown directly (in relation to HC coupling) that PKA is active in HCs. Furthermore, PKC has also been found to have a role in HC spinule dynamics (Weiler *et al.* 1991).

Interestingly, Pagh-Roehl *et al.* (1996) suggested that in addition to PKA and PKC, other as yet unidentified kinases could act as regulators of plasticity in the outer retina. In the present study, we have evaluated the role of tyrosine kinase (PKT) activity in control of cone PMMs and HC spinules, using genistein as a specific inhibitor (Akiyama *et al.* 1987).

## MATERIALS AND METHODS

Carp (*Cyprinus carpio*) with body length of 10-20cm were used for all the experiments. Fish were kept dark-adapted in an aerated cylindrical bucket containing cold tap water in a tank room under a strict 12:12h light: dark cycle. Experimental treatments were made on fish dark-adapted for at least 3h in their normal cycle. Fish were killed by decapitation, the eyes removed, hemisected and processed as follows: (1) *Dark-*

*adapted control.* Fully dark-adapted eyecups were each treated with a 50  $\mu$ l droplet of Ringer solution and kept in the dark for 30min. The Ringer solution contained the following (in mM): NaCl (102), KCl (2.6), NaHCO<sub>3</sub> (28), CaCl<sub>2</sub> (1), MgCl<sub>2</sub> (1) and glucose (5), pH 7.6 after bubbling with 95% O<sub>2</sub> + 5% CO<sub>2</sub>. (2) *Light-adapted control.* Dark-adapted eye-cups, treated with the Ringer solution alone, were exposed to steady white light (15mW/cm<sup>2</sup>) for 30 minutes. (3) *Genistein.* Dark-adapted eyecups were treated each with a 50  $\mu$ l drop of genistein (dissolved in the Ringer solution, giving a final, effective intra-retinal concentration of 18mM/mL), and then exposed to the same light stimulus (as in 2). Each experiment was repeated on three retinas, each from a different fish. Following the treatments, the eyecups were processed for light and transmission electron microscopy as described previously by Djamgoz *et al.* (1987). For each retina, the value of cone index (CI) was determined by measuring at least 100 cones (10 sections/3 blocks), whereby the total number of cones measured for each experiment was in the range of 300-380. Cone index was defined as x/y, where x = distance from the ellipsoid/outer segment border to the external limiting membrane (ELM); and y = distance from ELM to the ganglion cell layer, excluding optic nerve fibres (Haamedi & Djamgoz, 1996). The spinule/ribbon (S/R) ratio of HCs was calculated by dividing the total number of spinules by the total number of ribbons present in a given cone pedicle (Kirsch *et al.* 1991; Haamedi & Djamgoz, 1996). Pedicles from different sections were obtained from at least 3 different blocks for each retina. Measurements from cones and pedicles of all possible spectral subtypes were pooled indiscriminately.

All data were expressed as mean  $\pm$  SEM and analysed by student's t-test using the EPI-info statistical software package. P-values less than 0.05 were taken as significant.

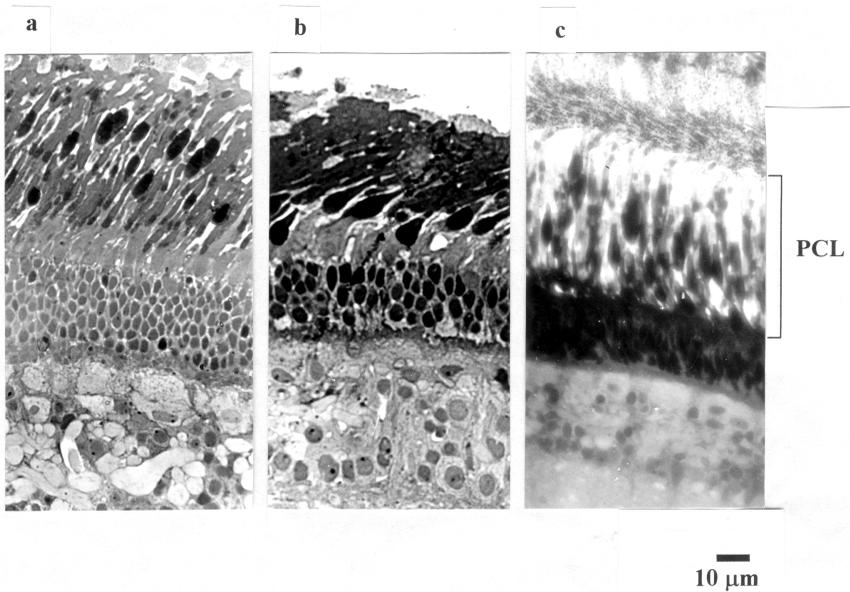
## RESULTS

### *Cone photomechanical movements.*

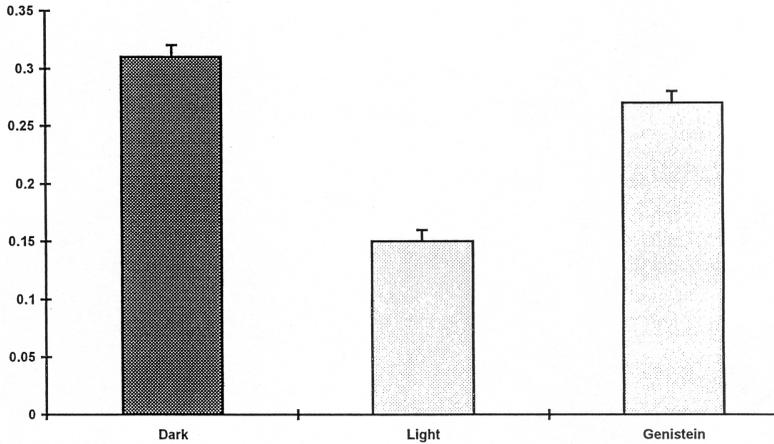
Figure. 1 shows typical light microscopic sections taken across the photoreceptor layer of retinae from each of the three experimental conditions used. The averaged data obtained are illustrated in Figure. 2. The control values of the cone index, taken from retinae treated with Ringer solution alone, were  $0.31 \pm 0.02$  (dark-adapted) and  $0.15 \pm 0.01$  (light-adapted). The difference was significant ( $p < 0.05$ ). When genistein was present in the eyecups during light adaptation, however, cones maintained a largely dark-adapted position, cone index reaching a value of only  $0.27 \pm 0.01$ . This was significantly different from values of cone index obtained from light- and dark-adapted eyecups under control conditions ( $p < 0.05$ ).

*Horizontal cell spinules.*

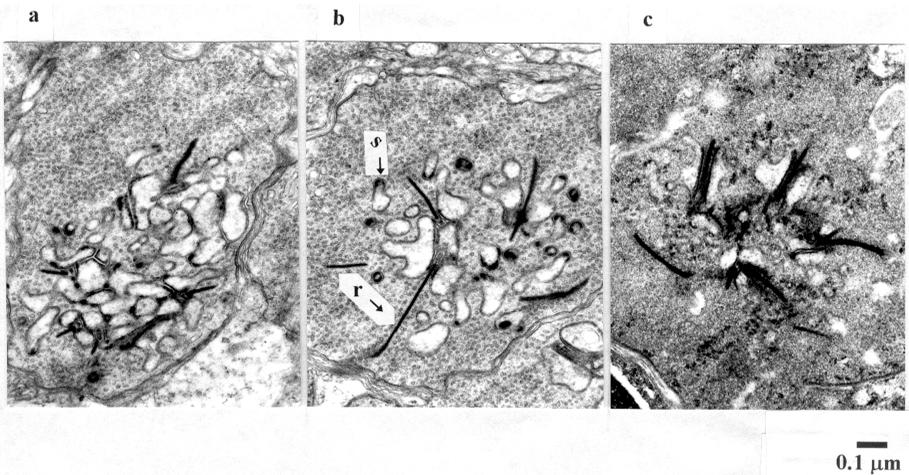
Typical electron micrographs showing HC spinules in cone pedicles obtained from retinae under the three experimental conditions are shown in Figure 3. Averaged data are illustrated in Fig. 4. The dark-adapted control value of S/R was  $0.58 \pm 0.01$ . During control light adaptation, the value of S/R increased to  $1.73 \pm 0.01$ , the change being significant ( $P < 0.05$ ). On the other hand, light adaptation in the presence of genistein gave an S/R value of  $0.60 \pm 0.01$ , i.e. the spinule content of cone pedicles remained largely in the dark-adapted situation. Student's t-test again showed that this was significantly different from the light- and dark-adapted controls ( $p < 0.05$ ).



**Figure 1.** Light microscopic sections of the carp retina across the photoreceptor layer (PCL) under different conditions: (a) Control dark-adapted. (b) Control light-adapted. (c) Light adapted in the presence of genistein ( $18 \mu\text{M}/ \mu\text{L}$ ). The scale bar ( $10\mu\text{m}$ ) applies to all the micrographs.



**Figure 2.** Quantitative data showing the mean values of the cone index (CI) corresponding to the three experimental conditions: Dark, dark-adapted control. Light-adapted control. Genistein, light-adapted in the presence of  $18\mu\text{M}/\mu\text{M}$  genistein. Error bars indicate standard errors.



**Figure 3.** Electron micrographs showing typical cone pedicels containing horizontal cell spinules, taken from retinæ under the three experimental conditions: (a) Control dark-adapted. (b) Control light-adapted. (c) Light-adapted in the presence of genistein ( $18\mu\text{M}/\mu\text{L}$ ). S = spinule. r = ribbon. The scale bar ( $0.1\mu\text{m}$ ) applies to all the panels.

## DISCUSSION

The present study would suggest, for the first time, that, in the carp retina, the light adaptive plasticity of both cones (photomechanical movements) and horizontal cells (spinules) is under the control of tyrosine kinase(s). This result generally complements other findings showing that PKT activity is involved in cellular and synaptic plasticity in the central nervous system (e.g. Beck *et al.* 1993; Catarsi *et al.* 1995). Furthermore, the additional protein kinase(s) predicted earlier to be involved in photoreceptor PMMs in the fish retina (Pagh-Roehl *et al.* 1996) could be a tyrosine kinase.

The presumed light adaptation-induced activation of PKT(s), in turn, may influence the cytoskeleton (directly or indirectly) e.g. by phosphorylation and bring about cellular and sub-cellular morphological changes (Djamgoz *et al.* 1996). Although the details of such mechanism(s) and especially the nature of the primary chemical messenger(s) involved are not yet clear, two possibilities in particular would be worth considering, as follows:

1. Nitric oxide (NO) has been found to be produced in fish retinae during light adaptation (Sekaran *et al.* 1999) and have a variety of light adaptive effects, including induction of cone PMMs and HC spinules (Greenstreet & Djamgoz, 1994) and HC (un)coupling (Lu & McMahon, 1997). Interestingly, PKT inhibitors have been shown to block NO synthesis (Rodriguez *et al.* 1994). So, one possible mode of action of PKT in the carp retina could be via control of NO production. Such an action could have additional secondary effects since the NO and dopamine systems have been shown to be interactive (Djamgoz *et al.* 1995b; Pottek *et al.* 1997).

2. Growth factor (GF) receptors are well known to have tyrosine kinase activity (e.g. Ullrich & Schlessinger, 1990; Heath, 1993). At present, little is known about the possible involvement of GFs in retinal light adaptation or functioning generally. Nevertheless, a variety of GFs and their receptors are known to exist in the retina (e.g. Perez & Caminos, 1995; Rickman & Brecha, 1995; Hallbook *et al.* 1996; Karlsson *et al.* 1998). Furthermore, light adaptation-induced turnover of GFs has been shown to occur in the avian and mammalian retina (Okazawa *et al.* 1994; Hallbook *et al.* 1996), consistent with a role in cellular/synaptic plasticity.

## CONCLUSION

In conclusion, tyrosine kinase activity is involved in the light-adaptive plasticity of the outer retinal neurones in carp. It seems, therefore, that the structural and corresponding functional dynamics of cone photoreceptors and HCs are under the control of an extensive system of neuromodulation and associated signal

transduction pathways. As far as the presumed PKT activity is concerned, however, further work is required to elucidate the link(s) with possible primary neurochemical(s).

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