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# Acidic fibroblast growth factor stimulates opsin levels in retinal photoreceptor cells in vitro

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It is demonstrated that newborn rat retinal photoreceptor cells can differentiate in monolayer culture, and synthesize de novo photoreceptor-specific proteins such as opsin. When maintained in serum supplemented medium on a laminin substrate, these cells survive for up to 3 weeks. The addition of acidic fibroblast growth factor stimulates an increase in the levels of opsin of 5–10-fold control values, and prolongs cell survival by up to 6 days.

Photoreceptor; Tissue culture; Growth factor; Opsin; Monoclonal antibody; (Bovine retina)

## 1. INTRODUCTION

In recent years it has become apparent that growth factors other than nerve growth factor have profound effects on neural cells. The fibroblast growth factors (FGFs), both acidic (aFGF) and basic (bFGF), formerly termed EDGF II and I respectively [1], exert a variety of influences on many neural primary cultures and cell lines. They stimulate proliferation, neurite outgrowth and cell survival in brain and PC-12 cells [2–5]. However, detailed molecular analysis of FGF effects is made difficult by lack of specific probes and appropriate models.

In the present study we demonstrate the versatility of the retina as a model permitting molecular analysis of FGF effects on neural growth and differentiation.

## 2. MATERIALS AND METHODS

### 2.1. *Monoclonal antibody*

Rho-4D2 has been described fully in previous reports [6,7]. It is specific for rod photoreceptor rhodopsin, and shows no cross-reactivity with other retinal proteins as determined by radioimmunoassay (RIA), Western blotting and immunoelectron microscopy [6,7].

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### 2.2. *Tissue culture and biological model*

Monolayer cultures of newborn rat retinal cells were established and maintained as described [8]. Cells were seeded at $10^5/cm^2$ onto laminin (1 $\mu g/cm^2$) coated surfaces.

aFGF was purified from bovine retina by heparin affinity chromatography and salt elution, purity being assessed by HPLC analysis [1]. It was used at 50 ng/ml [5], and was normally added at the time of culture initiation and renewed with each medium change. In one series of experiments cells were allowed to attach and develop for 2 days prior to aFGF addition. Semi-purified FGF (0.1 N acetic acid extract, or 0.1 N FGF) containing both aFGF and bFGF activities was also used, at a concentration producing maximal stimulation of epithelial cell division [1]. Heparin (Choay, France) was added at 10 $\mu g$/ml [5].

### 2.3. *Immunocytochemistry*

All antibody labelling of cells was performed as published previously [8].

### 2.4. *Radioimmunoassay*

RIA procedures were adapted from published methods [9]. Cultures were harvested by scraping at different time points from 3–21 days in vitro. Cells were pelleted and resuspended in phosphate-buffered saline (PBS), solubilized in Triton X-100 and aliquoted into microtitre assay plates. 20–100 $\mu g$ total protein was used for each aliquot. After drying, wells were preincubated with PBS containing 1% normal goat serum (NGS) and 1% bovine serum albumin (BSA) for 60 min, incubated with rho-4D2 (1 : 1000) for 60 min, washed and treated with

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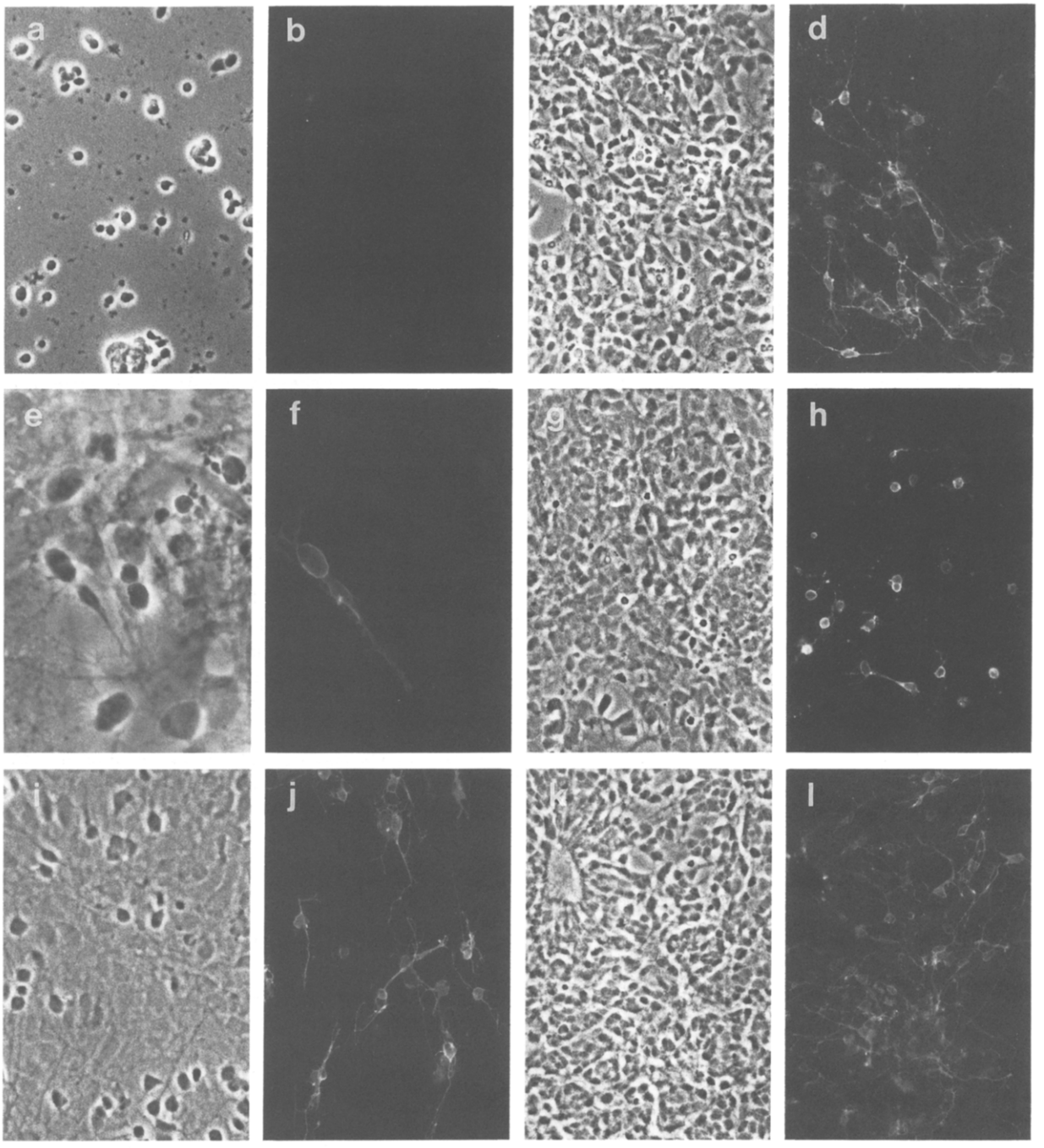


Fig.1. Rho-4D2 labelling of retinal cells cultured in the presence or absence of FGF. Phase contrast images of cells after (a) 0 (× 290), (c) 8 (× 290), (e) 11 (× 775) and (g) 15 days (× 290) in vitro without FGF, and (i) 13 (× 310) and (k) 18 days (× 310) in vitro with 0.1 N FGF. Fluorescence images of corresponding cells (b) 0, (d) 8, (f) 11, (g) 15, (j) 13 and (l) 18 days in vitro. Note brightly labelled cell bodies and fibres in both tests and controls.

$^{125}$I-goat anti-mouse IgG (Amersham; spec. act. 750–3000 Ci/mmol) (2–5 × $10^5$ dpm/well) for 60 min [9]. Following thorough washing, individual wells were cut out and counted in an LKB gamma counter.

## 3. RESULTS

### 3.1. *Immunocytochemistry*

Freshly seeded retinal cells do not express detectable opsin (fig.1a,b). Opsin containing cells appear at 2–3 days in vitro and increase in number and intensity of staining throughout the first 2 weeks in culture, eventually comprising 30–40% of rounded neuronal-like cells (fig.1c–f). In the third week cells begin to retract their processes and remain as rounded cell bodies until they die (fig.1g,h). Addition of 0.1 N FGF causes a slight delay in the initial appearance of opsin containing cells, but by the second week the number (25–40% of rounded cells), morphology and intensity of staining of labelled cells is similar in test and control cultures (fig.1i,j; table 1). FGF treated cultures survive for up to 6 days longer than parallel controls (fig.1k,l).

### 3.2. *Radioimmunoassay*

Solid phase RIA of control cultures exhibits either no detectable increases or slight increases in rho-4D2 binding with time, with a small maximum around 10–16 days (fig.2a–c). In cultures treated with 0.1 N FGF opsin levels gradually rise to attain maximal levels 5–10-fold greater than controls around 10 days in vitro (fig.2a). Opsin levels either remain elevated throughout the time examined or decrease slightly. Similar effects are seen with purified aFGF (fig.2b); addition of heparin to cultures does not further increase rho-4D2 levels (fig.2b). Differences in rho-4D2 binding between treated and untreated cultures are usually small prior to 4–6 days in vitro. Addition of aFGF 2 days after culture establishment also enhances rho-4D2 binding by an average of 5–10-fold control levels (fig.2c).

Table 1

Number of opsin-containing cells in the presence or absence of FGF

| Time in vitro (days) | % rounded cells labelled with rho-4D2 | |
|---|---|---|
| | Without aFGF | With aFGF |
| 5 | 0.2 (589) | 0.1 (580) |
| 7 | 1.6 (972) | 1.1 (611) |
| 9 | 12.4 (894) | 10.6 (692) |
| 13 | 28.5 (912) | 24.7 (688) |
| 15 | 39.8 (858) | 44.4 (792) |

## 4. DISCUSSION

The retina is a very useful model for studies of the central nervous system (CNS) owing to its neuroectodermal origin, accessibility, precise organisation and the availability of specific immunological markers [10]. Monoclonal antibodies have been instrumental in examining various aspects of photoreceptor development, structure and function [6–10]. In the present study we show that the retina is also an excellent tissue for examining growth factor effects on the CNS. This value depends upon the availability of such sensitive, well characterized probes and reproducible tissue culture models.

The presence of FGFs in the retina has long been known [1]. However, their putative role(s) in retinal cell biology is still poorly understood. FGFs exhibit angiogenic activities, and hence a role in retinal vascularization is postulated [11]. However, their stimulatory effects on a range of neural types [2–5] clearly suggest they may play additional roles in retinal neurobiology. Also, as the presence of FGF in photoreceptors and the regulation of its binding by ATP has been demonstrated [1], there is a clear precedent for FGFs having important roles in photoreceptor metabolism. The present study demonstrates that aFGF can stimulate the levels of an important photoreceptor-specific protein, opsin, and increase cell survival. bFGF has been reported as having no effect on retinal cell neurite extension or survival [4]. Although FGFs stimulate protein levels in glial cells [12] and neural cell lines [13], this is the first report of FGF stimulating specific protein levels in primary neurons.

The mechanism by which FGF stimulates opsin levels is not presently known. As immunocytochemical labelling of test and control cultures reveals no obvious differences in the number, morphology or intensity of staining of photoreceptors, the stimulation in opsin levels does not seem to be due to proliferation or increased fibre outgrowth [2–5]. It may involve an increased rate of

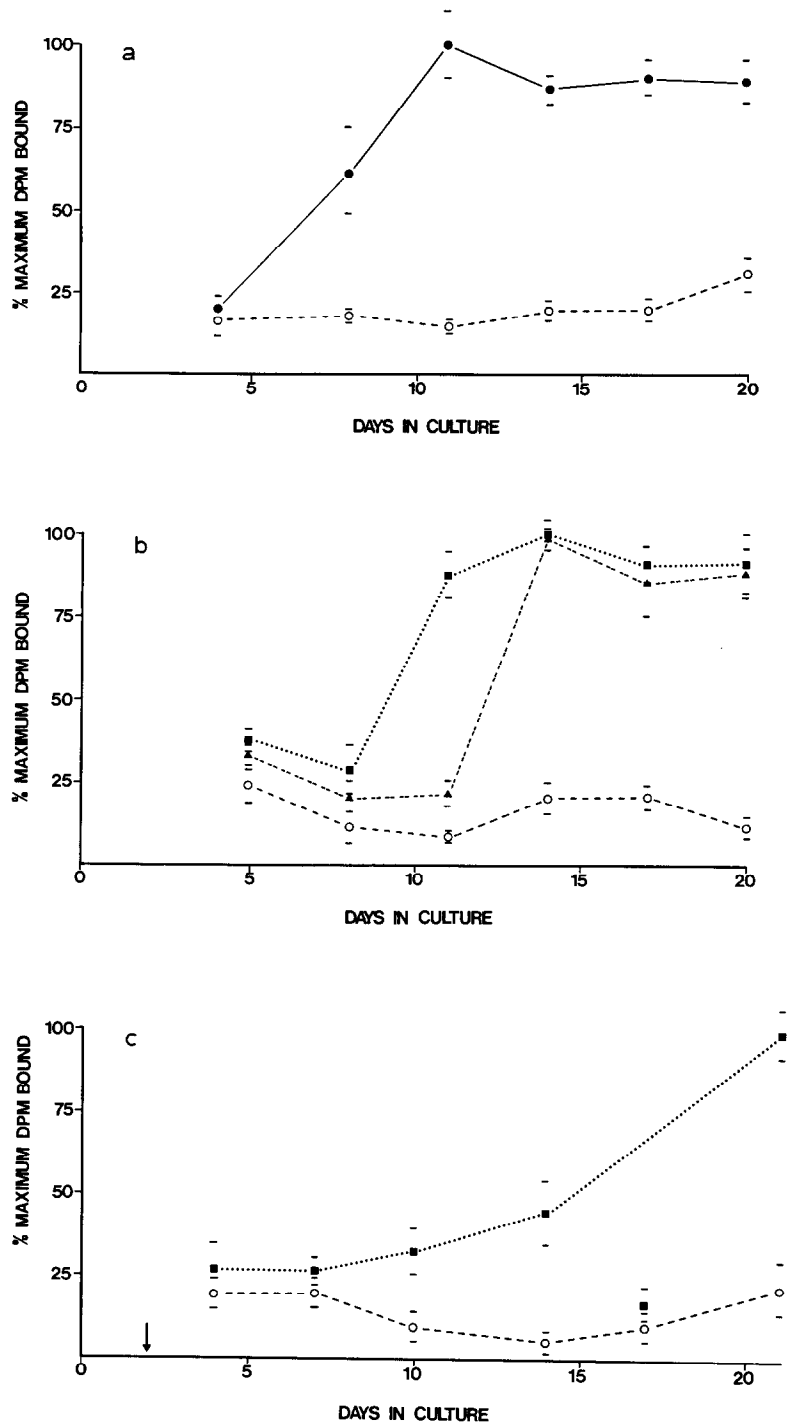


Fig.2. Radioimmunoassay of rho-4D2 binding to retinal cells cultured in the presence or absence of FGF. Values have non-specific binding subtracted and are expressed as mean ± SE of two separate experiments. (a) Untreated (○) and 0.1 N FGF treated (●) cultures; (b) untreated (○), aFGF treated (▲) and aFGF + heparin treated (■) cultures; (c) untreated (○) and aFGF treated (■) cultures, FGF added 2 days after cell seeding (arrow).

transcription or translation, or an increase in mRNA stability, which would be difficult to detect immunocytochemically. This possibility is being tested with the use of specific opsin cDNA probes. It is not simple differential adhesion as FGF treatment subsequent to cell attachment still elicits a response. Interestingly, other photoreceptor proteins show different time courses of responses corresponding to their in vivo appearance (in preparation). The choice of substrate is also important as FGFs may act synergistically with the extracellular matrix [14].

In conclusion, the present study demonstrates that aFGF stimulates the levels of photoreceptor-specific proteins in vitro. It will be of great interest to examine FGF effects on other retinal proteins and to investigate its action in degenerating retina. The retina forms an ideal model for analyzing the complex effects of growth factors on the differentiation of the CNS.

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