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Induction of the Stellate Configuration in Cultured Iris Epithelial Cells by Adenosine and Compounds Related to Adenosine 3′:5′-Cyclic Monophosphate

(cell dedifferentiation/cytoplasmic projections/morphological alterations)

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ABSTRACT **Adenosine, dibutyryl cyclic AMP, monobutyryl cyclic AMP, cyclic AMP, and 5′-AMP have a remarkable morphogenetic effect on cultured iris epithelial cells obtained from adult newt. They alter the broad undulating membrane of the cell into branching strands of cytoplasm, a configuration that has been named "stellate." Theophylline, a phosphodiesterase inhibitor also induces the stellate configuration. This transient morphological alteration is detectable by 30 min and becomes maximal 80 min after treatment. In the continued presence of the effective compounds the altered cells return to their normal shape, although the recovery period is variable. The morphological alteration of iris epithelial cells *in vitro* observed in the present experiment is reminiscent of that which occurs during the dedifferentiation phase in lens regeneration *in vivo*. These observations suggest that induction of the stellate configuration is relevant to the mechanism of dedifferentiation of newt iris epithelial cells during Wolffian lens regeneration.**

Removal of the lens from adult newts *Notophthalmus viridescens* leads to transformation of the dorsal iris epithelium into lens cells, a process known as Wolffian lens regeneration (1, 2). During this transformation the heavily pigmented iris epithelial cells depigment and lose their morphological identity and developmental specificity (3, 4). Morphological alterations of dedifferentiating iris epithelial cells *in vivo* have been studied by Dumont and Yamada (3): after lentectomy, intercellular spaces appear between cells and the normally smooth surface of the cell develops extensive projections; the slender tips of the projections contain melanosomes, and they become separated from the cells and are taken up by macrophages invading the iris epithelium. Repetition of such removal leads to complete loss of melanosomes, the characteristic organelles of differentiated iris epithelial cells. Currently, in our laboratory we are studying this dynamic process in individual iris epithelial cells cultured *in vitro*.

That adenosine 3′:5′-cyclic monophosphate (cAMP) plays a key role in morphogenesis has been demonstrated in several biological systems. Yokota and Gots (5) found that cAMP is essential for formation of flagella in coliform bacteria. Hsie, Puck, and their coworkers (6, 7) extensively studied the role of dibutyryl cyclic AMP (But_2cAMP) on morphological transformation of Chinese hamster cells *in vitro*. Prasad and Hsie (8) showed that But_2cAMP induces morphological differentiation of neuroblastoma cells *in vitro*. During melanosome dispersion in frog melanophores, cAMP mediates the action of the melanocyte-stimulating hormone (9, 10). This hormone, in addition to causing melanosome dispersion, produces a concomitant increase of cAMP in the melanophores (11). Furthermore, Novales (12) reported that when melanophores from embryonic newt in culture are treated with the hormone, the melanosomes move to the periphery of the cell and minute processes appear on the cell surface, suggesting that the hormone may produce an overall change in cell topography.

On the basis of the described effects of cAMP and the similarities of the cellular changes occurring during melanosome dispersion in melanophores and those that occur in iris epithelial cells during the dedifferentiation phase of lens regeneration, we decided to study the effect of cAMP and related compounds on iris epithelial cells cultured *in vitro;* we report here a remarkable morphogenetic effect of adenosine and compounds that are related to cAMP in iris epithelial cells.

Abbreviation: But, butyryl.

MATERIALS AND METHODS

Primary cultures of iris epithelial cells isolated from the dorsal iris epithelium of adult newt *N. viridescens* were used in all the experiments. Explants of dorsal iris epithelium stripped from their adherent stroma were placed in calcium- and magnesium-free phosphate-buffered saline (80%, pH 7.8) for 10–15 min. The explants were then treated with 0.25% trypsin solution for 30 min, after which the trypsin was removed and replaced with culture medium consisting of 50% L-15 medium, 40% water, 10% fetal-calf serum, and 2 ml of antibiotic–antimycotic solution per 100 ml (13). The tissues were then dissociated with a magnetic stirrer for 45–60 min. followed by repeated pipetting with a Pasteur pipette. The cells were then placed in 35-mm culture dishes (Falcon plastics) with or without a 22-mm^2 coverslip on the bottom of the dish. The cell cultures were maintained at room temperature 22.5 ± 0.5°. All tissue and cell culture reagents and medium were purchased from Grand Island Biological Co.

2- to 4-Week-old primary cell cultures of sparse population (200–500 cells per culture dish) were used in all the experiments. In each experiment, the culture was placed on an inverted microscope, and the iris epithelial cells were treated with the compounds shown in Table 1. The treatment lasted for 80 min unless otherwise indicated. Phase-contrast photomicrographs were taken at different time intervals. At the end of each experiment the medium was replaced with normal culture medium. All the chemical compounds were obtained from Sigma Chemical Co., unless otherwise indicated, and were dissolved directly into the culture medium.

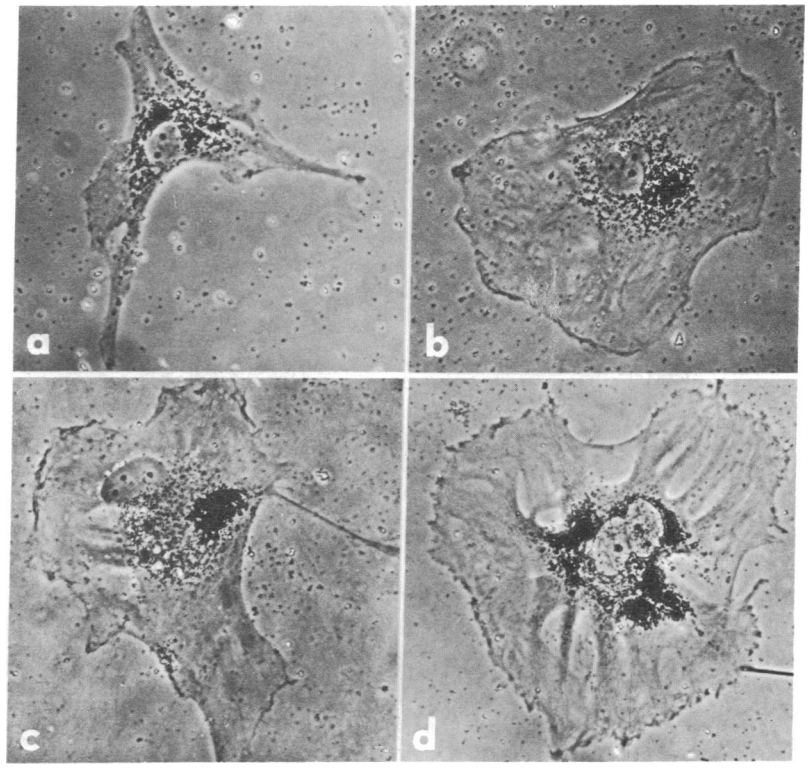


FIG. 1. Iris epithelial cells after 3 weeks (*a,b*) and 4 weeks (*c,d*) in culture. *Dark dots* outside the cells are free melanosomes discharged from IE cells. Magnification ×237.

RESULTS

Fig. 1 shows the morphology of iris epithelial cells after 3 and 4 weeks *in vitro*. The cells are widely spread, some are binucleate, and the majority of the nuclei contain more than one nucleolus. The remaining melanosomes are concentrated around the nucleus.

But$_2$cAMP (1–5 mM) induces a characteristic morphological alteration on the cellular surface, which can be detected by

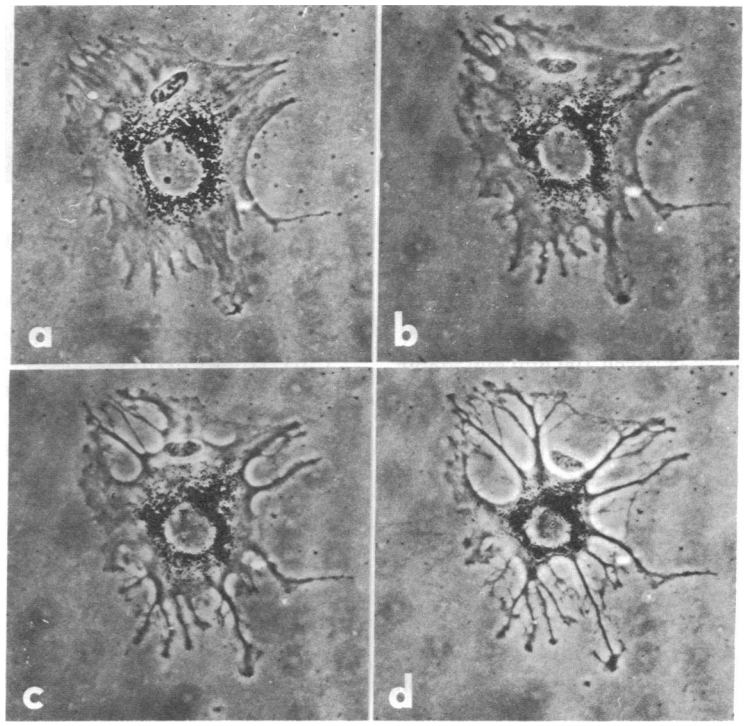
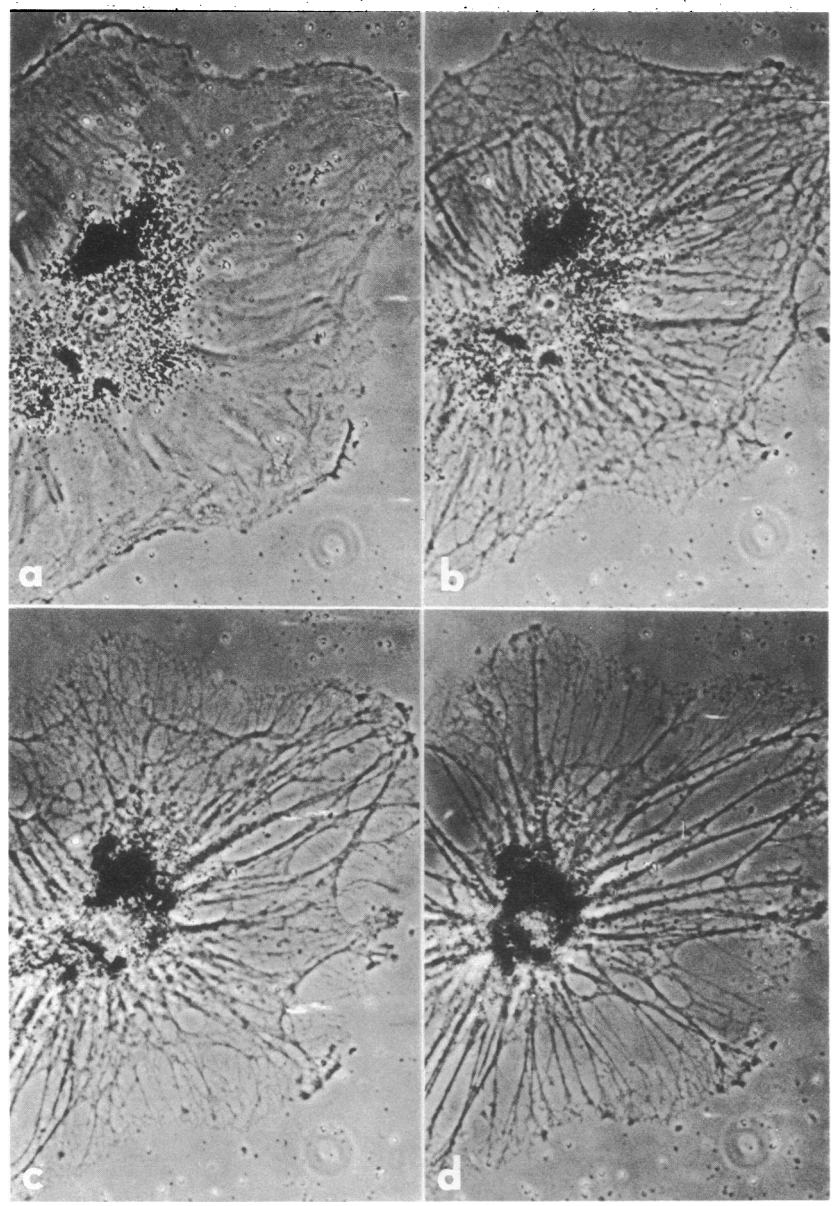


FIG. 2. Effect of 5 mM But$_2$cAMP on the morphology of iris epithelial cells. Photographs were taken at (*a*) 0, (*b*) 30, (*c*) 50, and(*d*) 80 min after addition of But$_2$cAMP. Magnification ×243.



30 min and becomes maximal by 80 min of treatment. One interesting aspect of the change in cellular configuration is that the effect is transient. In the continued presence of But_2cAMP in above indicated concentrations, the morphologically altered cells return to their normal shape between 160 min and about 12 hr after addition of the chemical agent. Fig. 2 shows that 5 mM But_2cAMP alters the broad undulating membrane of the cell into branching strands of cytoplasm. The original cell border corresponds to the position of the distal ends of the cytoplasmic strands and their branches. From the details of morphogenesis shown in Fig. 2, it is clear that the strands are formed not by growth of cytoplasmic processes extending out from the original cell border but by redistribution of the cytoplasmic material into a large number of strands. In general, the stellate cells appear essentially radially symmetrical, although sometimes the symmetry is disturbed by formation of cytoplasmic strands in only one sector of the cytoplasm. In eight experiments with 5 mM But_2cAMP, 14–37% of the cells were morphologically altered at the end of 80 min. No morphological alteration was observed in the untreated controls. Monobutyryl cyclic AMP (ButcAMP) at 1 mM induced the same morphological alteration as But_2cAMP. On the other hand, dibutyryl cyclic GMP (But_2cGMP), monobutyryl cyclic UMP (ButcUMP), and monobutyryl cyclic IMP (ButcIMP) tested at 1 mM failed to produce any effect.

Cyclic AMP had the same effect as But_2cAMP and ButcAMP when used at a concentration of 5 mM, but it was ineffective between 1 and 2.5 mM. Theophylline, an inhibitor of phosphodiesterase, was only effective at a relatively high concentration (5 mM). Only a weak effect was obtained with caffeine (5 mM), another inhibitor of phosphodiesterase. Sodium butyrate (10 mM), adenine (2.5 and 5 mM), and ribose (5 mM) were ineffective. Ribonucleosides like guanosine, uridine, and cytidine failed to produce any effect, and only adenosine induced the stellate configuration, even at concentrations as low as 0.01 mM. At 0.005 mM, adenosine was ineffective. The effect of adenosine is illustrated in Fig. 3. 5′-AMP was also effective, even at concentrations as low as

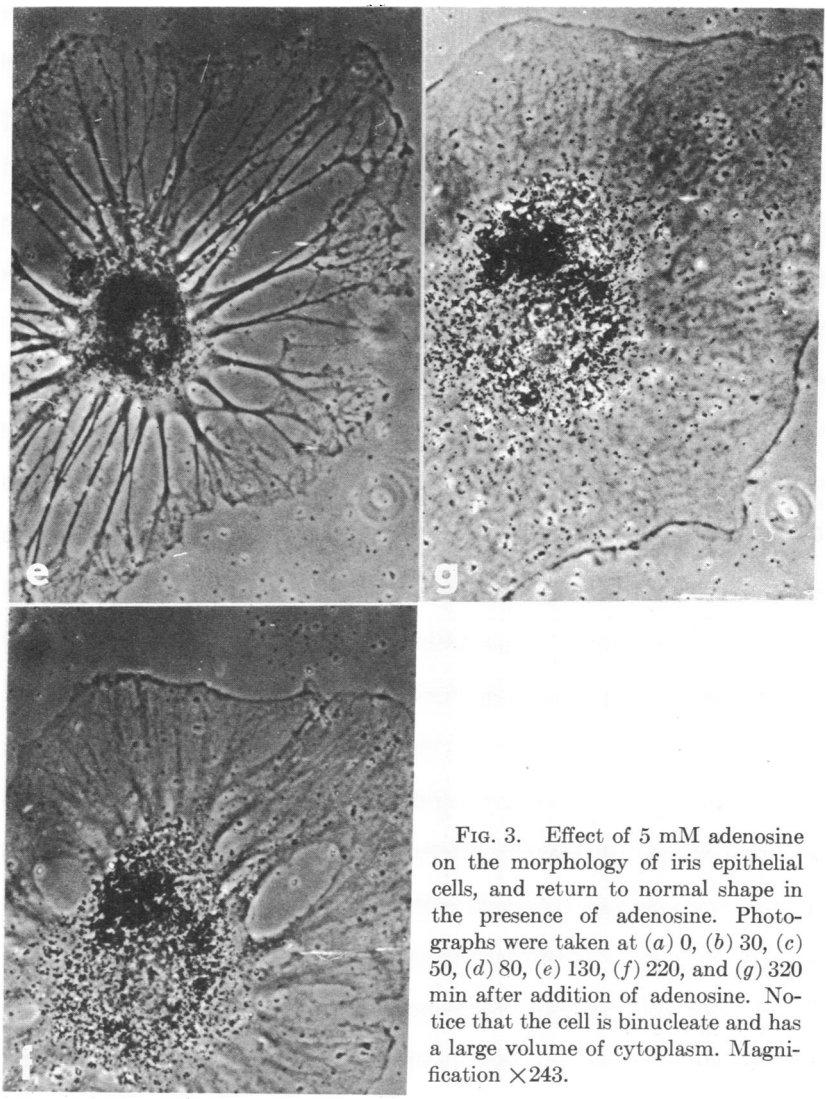


FIG. 3. Effect of 5 mM adenosine on the morphology of iris epithelial cells, and return to normal shape in the presence of adenosine. Photographs were taken at (*a*) 0, (*b*) 30, (*c*) 50, (*d*) 80, (*e*) 130, (*f*) 220, and (*g*) 320 min after addition of adenosine. Notice that the cell is binucleate and has a large volume of cytoplasm. Magnification ×243.

0.025 mM, although we have not determined its lowest effective concentration. Among all the compounds tested, adenosine seemed to be the most effective agent in causing the stellate configuration of iris epithelial cells. The L-15 medium used did not contain adenosine, 5′-AMP or ATP.

DISCUSSION

The results presented here demonstrate that compounds related to adenosine and cAMP can rapidly and effectively alter the morphology of cultured iris epithelial cells from adult newts. The observations that cAMP, its derivatives But_2-cAMP and ButcAMP, and theophylline, a compound that increases intracellular levels of cAMP due to its inhibitory effect on cAMP phosphodiesterase, are all effective and that sodium butyrate and other cyclic compounds tested are not, leads us to suspect that cAMP may be the agent responsible for induction of the stellate form. Induction of a similar morphological alteration has been observed in fetal-rat brain cells cultured in the presence of ButcAMP (14). The effect of adenosine and 5′-AMP could be due to their ability to enhance the intracellular level of cAMP, as was demonstrated in guinea-pig cerebral cortex slices (15). The minimum effective concentration of adenosine found by those workers coincides with that found in the present experiment. However, we cannot rule out the possibility that adenosine and 5′-AMP have a direct effect on the cell morphology. There is an earlier report of the effect of adenine nucleotides on cell-surface structure (16). The limited number of cultured iris epithelial cells available make it technically impossible to assess the precise role of either adenosine or 5′-AMP in the morphological alteration of iris epithelial cells *in vitro*. Such difficulties involve the quantitation of intracellular cAMP and its enzyme system, transport properties of the various effective compounds, and the activity of 5′-nucleotidase.

Puck, Waldren, and Hsie (7) have proposed that the morphological transformation of Chinese hamster ovary cells *in vitro* by But_2cAMP is related to the degree of organization of the microtubular–microfibrillar system of the cell. Electron microscopic studies by Porter, Puck, and Hsie and by Dumont and Hsie (both unpublished data) show that treatment of Chinese hamster ovary cells with But_2cAMP results in an increase in the microtubular population. The stellate configuration of the iris epithelial cells induced by adenosine and cAMP compounds is largely blocked when the cells are either pretreated with vinblastine at 10 μg/ml or simultaneously treated with vinblastine and one of the effective compounds. This ob-

TABLE 1. *Compounds tested on iris epithelial cells in vitro*

Compound	Concentration (mM)	No. of experiments	Induction of morphological alteration*
But$_2$cAMP	1.00–5.00	13	+
ButcAMP	1.00	5	+
But$_2$cGMP	1.00	5	−
ButcUMP	1.00	5	−
ButcIMP	1.00	5	−
Na-butyrate	10.00	2	−
cAMP	5.00	4	+
5′-AMP	0.025–5.00	5	+
Adenosine	0.01–5.00	14	+
Adenine	2.50–5.00	2	−
Ribose	5.00	3	−
Guanosine	1.00–2.50	2	−
Uridine	2.50	1	−
Cytidine	2.50	1	−
Theophylline	5.00	11	+
Caffeine	5.00	2	±

* + indicates induction of the stellate configuration; − indicates that the compound is ineffective in altering the cell shape; and ± indicates that induction of the stellate configuration by the compound was very weak.

servation leads us to suggest that microtubules may participate in the morphological alteration of iris epithelial cells.

In all our experiments we have observed that only part of the cell population responds to the compound used. One possibility is that only cells in certain period(s) of the cell cycle are responsive. The stellate configuration observed in the treated cells has certain morphological similarities with mitotic cells in culture; during cell division iris epithelial cells round up, and the broad cell membrane retracts toward the nucleus, forming slender strands connecting the cell body to the substratum. However, the morphological configuration induced by the effective compounds in our system is apparently not related to cell division; no cell division was observed in cells in which the stellate configuration was induced. Preliminary results from autoradiographic studies indicate that the S phase of the cell cycle is not the sensitive stage.

The morphological alteration of iris epithelial cells *in vitro* observed in the present experiment is reminiscent of that which occurs during the dedifferentiation phase in lens regeneration *in vivo*, in which extensive arrays of strands are produced (3). The fact that in the present *in vitro* system the strands have a radial, two-dimensional orientation, whereas *in vivo* the cells retain their proximo-distal polarity and their strands are three-dimensionally oriented, may be attributed to the difference between the environments of the cells. Free cells *in vitro* spread on the substratum, but cells *in vivo* are organized in an epithelium.

Thus the present results bring up the question of whether the cAMP level within the cell or the concentration of adenosine in the ambient milieu is involved in the dedifferentiation process that occurs in the iris epithelium during Wolffian lens regeneration (3, 4). According to our observations, induction of the stellate configuration *in vitro* under the present experimental conditions does not lead to detectable discharge of cytoplasmic components, which would be expected to occur if the process were the essential mechanism of dedifferentiation However, when iris epithelial cells are cultured continuously in the presence of 0.5 mM adenosine, the tips of cytoplasmic strands are pinched off and become separated from the rest of the strands when the latter are retracted to the cell body. Melanosomes are often included in the shedding process. These recent observations suggest that the induction of the stellate configuration demonstrated in the present experiment has important relevance to the mechanism of dedifferentiation.

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