Melanocortins stimulate proliferation and induce morphological changes in cultured rat astrocytes by distinct transducing mechanisms

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Melanocortin family of peptides is processed from a common precursor glycoprotein, pro-opiomelanocortin (POMC) of 31 kDa and includes adrenocorticotropic hormone (ACTH) and the melanotropins, α-, β-, γ-melanocyte stimulating hormone (MSH). All melanocortins share the heptapeptide sequence 4-10 of ACTH. In the central nervous system (CNS) and peripheral nervous system (PNS) these peptides have been shown to affect diverse and complex networks (for review, see Eberle13). These include regulating behavior in test animals,11,64 affecting memory and learning,64 inducing lacrimal secretion in the rat14,27,31, facilitating recovery of motor performance and nerve regeneration in the PNS9,10,43,57 and CNS61-63, causing transmitter release in motor nerve terminals28 and having therapeutic importance in peripheral nerve disease22.

Immunocytochemical studies have shown cell bodies containing melanocortins within the arcuate nucleus and in the dorsolateral hypothalamus which send axonal projections to many brain areas, including the limbic midbrain structures.42-59. More defined sites of melanocortin action had not been identified until recently, when specific, high affinity binding sites for [125I]ACTH1-14 in brain membrane preparations40 and receptors which recognize both ACTH and α-MSH by autoradiography in the adult rat brain have been described43. However, the specific cells or mechanisms by which melanocortins modulate their effects on the CNS are still unclear.

MSH and ACTH receptor pathways in melanogenic tissue and in adrenal cortical cells, respectively, are mediated by G-proteins, through adenylate cyclase (AC) and the cAMP cascade19,32. Uniquely, MSH2°,3a,45 and ACTH7 binding to their respective receptors in melanoma, lacrimal and adrenal cells is regulated by Ca2+ ions. Similar transduction mechanisms regulating different cell specific responses are expected to exist in melanocortin sensitive cells in the mammalian brain. Cyclic AMP stimulation in neonatal rat brain primary cell cultures55 and in more purified astroglial cells15 suggested the presence of a specific melanocortin receptor within the brain astroglial cell population. In the vertebrate adult and immature CNS, astrocytes are one of the ma-
jor cell types involved in regulating axonal growth or regeneration after injury (for review see Schwartz et al.46). Therefore the behavioral and developmental actions of melanocortins may in part be mediated by astroglial cells.

Cyclic nucleotides, in general, influence growth of various cell types. In many tissues cAMP appears to be a negative growth regulator. Long term culturing of M2R melanoma cells with phorbol esters increased proliferation which was blocked by the elevation of cAMP by forskolin or β-MSH17. In other cellular models, such as avian chondroprogenitor cells, cAMP has been reported to have a mitogenic effect18.

Astrocytes in culture undergo marked morphological and biochemical differentiation. Two specific markers of astrocytes, glial fibrillary acidic protein (GFAP) and glutamine synthetase (GS), increase by several fold both in vivo and in vitro suggesting that maturation of this cell type can be achieved in vitro25. Astrocytes in culture appear to change their morphology to round, short process type cells. Cyclic AMP derivatives (such as dibutyryl cyclic AMP), forskolin and neurotransmitters, which increase cAMP levels in astrocytes, accelerate both morphological and biochemical astroglial cell differentiation29,39.

This study was aimed at identifying particular cell-specific responses elicited by melanocortins in well defined homogeneous primary astrocyte cultures. Employing a [methyl-3H]thymidine incorporation assay and monitoring morphological changes40, we report here that melanocortins influence astrocyte proliferation and differentiation by distinct transducing mechanisms which are differentially expressed throughout the 3 weeks of development in culture.

MATERIALS AND METHODS

[2,3H]Adenine (9.77 Ci/mmol) was obtained from Kamag, Israel. [Methyl-3H]thymidine (6.7 Ci/mmol) was from New England Nuclear, Boston, U.S.A. α-Melanocytostimulating hormone (α-MSH), atropine, carbacebol, isoproterenol (ISO), EGTA, gentamicin sulfate, isobutylmethylxanthine (IBMX), poly-L-lysine, bovine pancreatic trypsin were from Sigma, St. Louis, MO and Forskolin were supplied by the departmental animal breeding center. Dulbecco’s Modified Eagle Medium (DMEM) and L-15 medium were supplied by Gibco, England. Foetal Calf Serum (FCS) and L-glutamine were purchased from BioMakor, Israel. Male Wistar neonatal rats (1-2 days old) were supplied by the departmental animal breeding center.

Primary astroglial cell cultures were prepared from the cerebral cortex of 1-2-day-old rats as described by McCarthy and de Vellis30 with modifications of Wolswijk and Noble80. The tissue was dissected to 1-2 mm3 sections in L-15 medium, filtered through a nylon mesh of 220 μm and incubated in 2 ml Ca2+-, Mg2+-free Dulbecco’s modified Eagle’s medium (DMEM-CMF) containing 0.5 mM EDTA and trypsin (300 i.u. ml−1) for 10 min at room temperature. Trypsinization was terminated by adding DMEM containing 10% FCS, and the cells were sedimented at 1000 g for 5 min. The pellet was washed to remove debris and resuspended in DMEM supplemented with 2 mM L-glutamine, 10% FCS and 15 μg/ml gentamicin (DMEM-FCS). Dissociated cells were plated at a density of 2 brains per 75 cm2 NUNC tissue culture flask pre-coated with poly-l-lysine (20 mg/ml) and incubated at 37°C in a humidified incubator with 5% CO2-95% air. Culture medium was replaced with fresh DMEM-FCS the following day and then 3 times each week until cells were utilized. Two days prior to the desired age of culture, i.e. on day 5, 12 and 19, for 7, 14 and 21 day experiments, respectively, the layer of nonadherent cells growing on top of the flat monolayer was removed by shaking the flask for 8 h at 37°C on a rotary platform (150 revs min−1). The medium containing the loosely bound process bearing cells was removed and normally discarded except for experiments in Fig. 1. The remaining adherent astrocytes in the flask were to be recovered for 1 day, then briefly, medium removed and then harvested (0.1% trypsin for 15 min at room temperature). After centrifugation, the pellet was resuspended in DMEM-FCS and seeded in the desired concentration to poly-l-lysine precoated 18 mm glass coverslips (105 cells/μl for immunofluorescence), 24-well Nunc plates (3 × 105 cells/well for [methyl-3H]thymidine incorporation studies, ~106 cells/well for cAMP accumulation studies) or 35 mm petri dishes (for microscopy) as described in the individual experiments. Each culture was tested for GFAP immunoreactivity. Most cultures routinely tested >90% GFAP positive; the lowest degree of homogeneity permitted for experiments was 85% GFAP positive.

Immunocytochemical staining

The culture’s cellular homogeneity was determined by indirect immunofluorescence labelling45. Cells, plated to glass coverslips in 35 mm culture dishes, were fixed with 5% glacial acetic acid in ethanol for 20 min at 4°C, followed by permeabilization with cold acetone (−20°C) for 5 min. The coverslips were then washed 3 times with Hank’s Balanced Salt Solution (HBSS containing 4% FCS (HBSS-FCS)). Incubation with GFAP monoclonal antibodies (mouse IgG1, 1:100 in HBSS-FCS) was carried out at room temperature for 30 min. Coverslips were washed 3 times with HBSS-FCS followed by a 30 min incubation at room temperature with rabbit anti-mouse IgGs conjugated to tetramethylrhodamine isoioxocyanate (RAM-TRITC, 1:50 in HBSS-FCS). The coverslips were subsequently washed 3 times in phosphate buffered saline solution (PBS), once in double distilled water (DDW), and fixed on a microscope slide in a mixture of 45% glycerol, 45% ethanol, and 10% PBS. Immunolabelled cells were photographed using a Zeiss Universal microscope equipped with phase contrast and fluorescence optics. Purity of the culture is expressed in terms of GFAP positive cells per 100 cells counted.

Measurement of cAMP accumulation

Intracellular [3H]cAMP accumulation in cell monolayers was measured using the [2-3H]adenine prelabelling method described by Salomon44. Briefly, cells were incubated with [2-3H]adenine (5–15 μCi/ml) in DMEM for 2 h at 37°C in a humidified 5% CO2 atmosphere, and the wells were then washed with 1 ml of DMEM to remove excess [2-3H]adenine. Cyclic AMP accumulation studies were conducted in 0.5 ml of DMEM containing 0.1 mM IBMX, 100 μg/ml BSA, and the appropriate stimulants at the concentrations indicated. Incubation at 37°C for the indicated time, was terminated by aspiration of the assay medium and addition of 1 ml 2.5% perchloric acid containing 0.1 mM cAMP. The clear perchlo-
ric acid extract was neutralized with KOH and subjected to double column (Dowex 50/alumina) chromatographic fractionation to determine the amount of $[2^{-3}H]cAMP$ formed. Intracellular accumulation of $[2^{-3}H]cAMP$ was calculated as a percentage of total $[2^{-3}H]adenine uptake/well. Data was expressed as mean fold increase $\pm$ S.E.M. of 3 separate wells similarly treated (triplicate determination) over nonstimulated controls.

**Measurement of [methyl-3H]thymidine incorporation**

Incorporation of [methyl-3H]thymidine into astrocyte DNA was measured as described by Pandiella et al. 37, with slight modifications. Medium of 7-, 14-, or 21-day-old cultures was changed to the same medium without serum but supplemented with 0.1% BSA (essentially fatty acid free) for 48 h. More than 90% of the cells survived serum-free conditions, as determined by trypan blue exclusion. The test substances were then added for 24 h, and 1 $\mu$Ci/ml [methyl-3H]thymidine was included for the last 6 h of the incubation at 37°C under an atmosphere of 5% CO2/95% O2. Cells were washed twice with cold PBS and nucleic acids and proteins were then precipitated with 7.5% trichloroacetic acid (TCA) for 30 min at 4°C. Acid insoluble material was washed twice with 0.5 ml ethanol, then solubilized in 0.3 ml of 1.0 NaOH. Radioactivity was measured by beta-scintillation counting and the means from 3 separate wells similarly treated (triplicate determination) were calculated $\pm$ S.E.M.

**Determination of morphological changes**

The shape of the intermediate filament cytoskeleton was monitored by GFAP indirect immunofluorescence microscopy. Seven-, 14- or 21-day-old astrocyte cultures were incubated with or without test substances in DMEM-FCS. Cells were monitored by phase contrast microscopy during this time. After 24 h, cells were fixed in ethanol:acetic acid (95:5 v:v), for 20 min at 4°C, permeabilized with acetone at $-20^\circ$C for 5 min and treated with anti-GFAP antibodies, as described above.

**Statistical analysis**

All statistical tests were carried out using the STATVIEW 512 program in a Macintosh personal computer. Student's t-test or one way analysis of variance (ANOVA) followed by the Fisher's Least Significance Difference (LSD) test were used to determine significant ($P < 0.001$) differences over control means.

**RESULTS**

**Regulation of cAMP levels by melanocortins**

In order to examine whether the cAMP response to melanocortins, in primary brain cultures, is indeed confined to astrocytes we determined the melanocortin response in the nonadherent cells and in the purified astrocytes derived from the heterogeneous brain cell population (Fig. 1). The melanocortins induced a significant increase in cAMP in the purified 21-day-old astrocytes (Fig. 1A). ACTH$_{4-10}$ (0.1 mM), ACTH$_{1-17}$ (100 nM) and 4,7-$\alpha$-MSH (100 nM) raised the cAMP levels by 14-, 9- and 11-fold, respectively, over nonstimulated controls. Isoproterenol (1 $\mu$M), used as a positive control in these experiments, stimulated cAMP accumulation in the purified astrocytes to a much higher extent (~40-fold). The nonadherent mixed population of cells (separated by shaking and usually discarded) were essentially nonresponsive to melanocortin stimulation, but exhibited a slight response to isoproterenol (Fig. 1B). In order to examine the melanocortin response in the two cell populations at higher sensitivity we also measured the response to melanocortins in the presence of forskolin. We tested the effect of forskolin (100 nM) alone and in combination with melanocortics on the 21-day-old astrocyte cultures and observed a synergistic response (Fig. 1A). However, in spite of the presence of forskolin, the melanocortins failed to stimulate cAMP accumulation in the nonadherent mixed cells (Fig. 1B). These results indicate that even under more sensitive assay conditions a cAMP response to melanocortins in the nonadherent cells is undetectable. The same synergistic effect of forskolin and melanocortins was also observed in 14-day-old astrocytes (Fig. 2) and in 7-day-old astrocytes (not shown). When culturing several regions of the rat brain, it appeared that melanocortins evoked the highest cAMP response from the cerebral cortex which included the astrocytes of the limbic system (data not shown).

Since astrocytes undergo spontaneous maturation in culture, we next examined whether the responsiveness to melanocortins varied over a period of 21 days. The
The synergistic effect of forskolin on melanocortin stimulated cAMP accumulation in 14-day-old cultured cortical astrocytes. Cells were incubated with melanocortins (100 nM), in the absence or presence of forskolin (FO) (100 nM) for 10 min and [2-^3H]cAMP accumulation determined under standard conditions. (% conversion to [2-^3H]cAMP in absence of stimulants (None = 0.055). All other details were as described under Materials and Methods. Values are means ± S.E.M. of triplicate determinations. Data are representative of 4 similar experiments with comparable results.

cAMP response to melanocortins and isoproterenol gradually increased with the age of the culture and varied for the different melanocortins used (Fig. 3). All tested peptides (except for ACTH4-10) were used at a final concentration of 1 μM to elicit maximal cAMP response. Stimulation of the cells was seen as early as 7 days in culture, with β-MSH, α-MSH, ACTH1-17, and 4,7-α-MSH eliciting a cAMP increase over nonstimulated controls of 1.2-, 1.2-, 1.6- and 2.2-fold, respectively. ACTH1-24 induced a significantly higher response at 7 days (3.8-fold increase) and remained a most potent agonist at all ages tested (Fig. 3, upper panel). The cAMP response to all tested peptides was greatest at 21 days in culture, the longest period tested in these experiments (Fig. 3). By 21 days, it appears that ACTH1-24 and 4,7-α-MSH are similar in their ability to increase cAMP levels, followed (in decreasing order) by ACTH1-17 and α- and β-MSH. The behaviorally active heptapeptide fragment, ACTH4-10 at 0.1 mM11,55, also showed the highest degree of stimulation at 21 days, and the extent of stimulation was about twice that seen with the other melanocortins. Even at 7 days, ACTH4-10 was formidable in elevating the cAMP in these cells to levels comparable to those induced by isoproterenol.

Induction of astrocyte proliferation by melanocortins

It was hypothesized that the cAMP response to melanocortins in astrocytes might mediate a cell specific response such as growth. We therefore examined the possible mitogenic effect of these peptides using [methyl-^3H]thymidine incorporation into DNA as an index of cell proliferation. Following serum deprivation for 48 h, 7, 14, and 21 day astrocyte cultures were exposed to the various melanocortins (Fig. 4). TriPLICATE wells containing 10% FCS were included with each experiment as a positive growth control. Melanocortins enhanced DNA synthesis only in the 7-day-old cultures. ACTH1-24 (100 nM), 4,7-α-MSH (100 nM) and ACTH4-10 (0.1 mM) elicited a 1.7-, 1.9-, and 1.8-fold increase in [^3H]thymidine uptake over the nonstimulated control, respectively. In contrast, none of the peptides tested induced proliferation in 14 and 21 day cultures. FCS (10%) elicited a 5-8-fold increase and carbachol (1 mM) a 2-fold increase in [methyl-^3H]thymidine incorporation irrespective of the culture age, i.e. at 7-, 14- and 21-day-old cultures (Fig. 4). The proliferative response to carbachol, first reported by Ashkenazi et al.4 was abolished by the addition of 10 μM atropine, suggesting muscarinic acetylcholine receptor involvement. These results indicate that cultured cortical astrocytes were capable of proliferation under a mi-
**TABLE I**

Effects of melanocortins on DNA synthesis and cAMP accumulation in 7 and 14 day cortical astrocyte cultures

<table>
<thead>
<tr>
<th>Stimulants</th>
<th>7 Days [Methyl-3H]thymidine incorporation</th>
<th>14 Days [Methyl-3H]thymidine incorporation</th>
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<tr>
<td></td>
<td>(c.p.m./well ± S.E.M.)</td>
<td>(fold)</td>
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<tr>
<td>None</td>
<td>11382 ± 302</td>
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<tr>
<td>10% FCS</td>
<td>72084 ± 2040*</td>
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<tr>
<td>ACTH₁₋₂₄ (100 nM)</td>
<td>22791 ± 972*</td>
<td>2.0</td>
</tr>
<tr>
<td>ACTH₁₋₁₇ (100 nM)</td>
<td>21610 ± 526*</td>
<td>1.9</td>
</tr>
<tr>
<td>ACTH₄₋₁₀ (0.1 mM)</td>
<td>20997 ± 289*</td>
<td>1.8</td>
</tr>
<tr>
<td>4,7-α-MSH (100 nM)</td>
<td>21374 ± 985*</td>
<td>1.9</td>
</tr>
<tr>
<td>α-MSH (100 nM)</td>
<td>22525 ± 699*</td>
<td>2.0</td>
</tr>
<tr>
<td>β-MSH (100 nM)</td>
<td>22206 ± 300*</td>
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</tr>
<tr>
<td>ISO (1 μM)</td>
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<table>
<thead>
<tr>
<th></th>
<th>cAMP (fold)</th>
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<tr>
<td>None</td>
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<tr>
<td>10% FCS</td>
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<td>ACTH₁₋₂₄ (100 nM)</td>
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<td>ACTH₁₋₁₇ (100 nM)</td>
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<td>ACTH₄₋₁₀ (0.1 mM)</td>
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<tr>
<td>4,7-α-MSH (100 nM)</td>
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<td>α-MSH (100 nM)</td>
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<tr>
<td>β-MSH (100 nM)</td>
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</tr>
<tr>
<td>ISO (1 μM)</td>
<td>19.1</td>
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Data for thymidine incorporation is compiled from 4 experiments. Values for [methyl-3H]thymidine incorporation are means ± S.E.M. for 3-6 individual determinations. cAMP was determined as described in Materials and Methods. *P < 0.001 over basal determinations (one way analysis of variance and Fisher LSD).
Fig. 6. Morphological changes induced in 14-day-old primary rat astrocytes by ACTH$_{1-17}$. Cells were cultured for 14 days in Dulbecco's modified Eagle's medium (DMEM-FCS), and incubated for 24 h in the presence of ACTH$_{1-17}$ (100 nM) or forskolin (100 nM). Incubation was terminated by washing with phosphate buffered saline (PBS) and cellular fixation followed by immunofluorescent labeling with anti-glial fibrillary acidic protein (GFAP) antiserum as described in Materials and Methods. A: nonstimulated control cells, flat, polygonal morphology; B: incubated in the presence of ACTH$_{1-17}$, cells develop a process-bearing morphology; C: incubated in the presence of forskolin, cells develop similar process-bearing morphology.
to FCS is unknown, the proliferative effect of carbachol in astrocytes was reported to correlate well with the degree of activation of phosphoinositide hydrolysis. To evaluate whether the proliferative effect of melanocortins in these cultures is related to their ability to stimulate cAMP accumulation, we incubated the cells with other stimulators of cAMP under similar experimental conditions. Isoproterenol (100 nM) and forskolin (100 nM) failed to elicit an increase in [methyl-3H]thymidine incorporation in either 7 or 14 day cultures when included in the incubation medium (Fig. 5). In addition, the positive growth response to ACTH1-17 (100 nM) in 7-day-old cultures was abolished by simultaneous incubation with either forskolin (100 nM) or isoproterenol (100 nM). In agreement with the results (Fig. 4), only the 7-day-old cells responded to 4,7-α-MSH (100 nM) and ACTH1-17 (100 nM) by increasing [3H]thymidine incorporation. A 1- and 1.8-fold over nonstimulated controls, respectively.

All of the melanocortins tested increased [methyl-3H]thymidine incorporation in 7 day astroglial cells (Table 1). There appeared to be no significant difference in the extent of proliferation induced by the various melanocortins tested irrespective of their ability to stimulate significantly different cAMP levels. In particular, β-MSH and α-MSH, which are weak stimulants of cAMP production in 7 day cultures, were as effective as ACTH1-24 in enhancing [methyl-3H]thymidine incorporation. The melanocortin peptides, in general, increased [methyl-3H]thymidine incorporation with a pharmacological profile which did not parallel that observed for their ability to stimulate cAMP accumulation. In 14-day-old cultures, no effect on DNA synthesis was observed although melanocortins elevated cAMP levels to a significantly higher extent.

**Morphological changes in astrocytes as induced by melanocortins**

Studies by others have described morphological differentiation in cultured astrocytes upon administration of cAMP elevating agents or cAMP derivatives. Further studies were therefore conducted to examine the influence of melanocortins via adenylate cyclase on astrocyte morphology using GFAP as a marker for changes of the intermediate filament organization. Astrocytes (14 days) were incubated in the presence of ACTH1-17 (100 nM), forskolin (100 nM), isoproterenol (100 nM) or vasopressin (1 μM). Morphological changes were observed by light microscopy as soon as 2 h after commencing incubation with isoproterenol or forskolin. ACTH1-17 as a representative of melanocortins induced morphological changes after 20–24 h. In the presence of ACTH1-17, the 14-day-old astroglia converted from polygonal (Fig. 6A) to process bearing cells (Fig. 6B) that resemble forskolin (Fig. 6C) and isoproterenol (data not shown) treated astrocytes. Similar morphological changes were observed in the 7- and 21-day-old astrocyte cultures (data not shown). Vasopressin did not elicit any observable morphological response (data not shown). This peptide served as a control as it was shown to be ineffective in elevating cAMP levels in these cells. These data indicate that the morphological differentiation of cultured astrocytes can be influenced by melanocortins throughout the culture period and that this effect appears to be mediated by cAMP.

**DISCUSSION**

Melanocortins appear to specifically affect primary cultures of purified rat astrocytes and the magnitude of the response in terms of cAMP production seems to increase with the age of the cells in culture. Our findings support the hypothesis that putative melanocortin receptors reside on the astrocyte population of rat brain cells in culture, possibly in situ as well. In addition, our results suggest that melanocortins are regulating two cell specific responses in astrocytes via different signal transduction pathways: proliferation and morphological differentiation, both of which do not develop simultaneously.

The melanocortins failed to elicit a cAMP response in the heterogeneous nonadherent layer of cells derived from whole brain primary cultures, which consist of putative O-2A progenitor cells, fibroblasts, type-2 astrocytes, and oligodendrocytes. These cells were not typed with specific antigenic markers and therefore, their exact identity was only evaluated on the basis of morphology. Even under more sensitive assay conditions by inclusion of forskolin, which potentiates hormone stimulated cAMP accumulation in various cell types and has been shown by us to increase the sensitivity to MSH by a factor of 40 and to increase maximal response level by 3-fold in mouse M2R melanoma cells, a cAMP response to melanocortins in the nonadherent cells is undetectable. The response to isoproterenol, however, indicates the viability of these cells which are known to express β-adrenergic receptors. However, since the cells in this study were selected on the basis of their cAMP response, we cannot exclude the possibility that melanocortins may stimulate other brain cell types via alternative signal transduction mechanisms.

The melanocortins have been shown to affect complex behavioral functions, as well as fetal brain development. Various studies attempted to correlate specific brain regions with the induced behavioral effect (for review see De Wied and Jolles). In our studies, the cerebral cor-
The possibility that melanocortins may afford their pleiotropic effects in cells by effector enzymes other than adenylate cyclase has been considered 5'13'26'49. However, to the best of our knowledge, no direct demonstration has been reported concerning the activation of inositol lipid specific PLC by melanocortins.

The results presented are compatible with the presence of two melanocortin receptor subtypes which may employ separate signal transducing pathways and a differential pattern of receptor expression associated with the development of the astrocytes in vitro. In the young cultures (7 days), both types might be present as indicated by both the proliferative and cAMP responses. As the cells mature, the relative ratio between the two types of receptors might change, leading to higher cAMP levels, and loss of the proliferative effect. The pharmacology of these putative melanocortin receptor subtypes is certainly different (Fig. 3 and Table I). While it appears that the melanocortins are equipotent in stimulating the effector system controlling proliferation, their relative potencies in regulating cAMP accumulation are significantly different. The efficacies of the different melanocortins on these putative receptor subtypes and consequently the resulting cellular responses may be complex. For example, ACTH4-10 can elevate cAMP levels to nearly those attained with isoproterenol, yet unlike isoproterenol, can induce astrocyte proliferation in 7-day-old cultures. This may be explained by a difference in the type of receptor activated or the peptide fragment’s relative potency on the putative melanocortin receptor subtypes. Melanocortins may also be working indirectly, stimulating the production of an additional substance responsible for the mitogenic effects observed. In support of this receptor subtype hypothesis it has been shown, using crosslinking techniques, that two putative MSH receptors appear to reside on M2R melanoma cells18,52. Multiple receptors regulating parallel transducing pathways have also been suggested for ACTH41 and other hormones such as glucagon58.

Our results suggest that the control of adenylate cyclase by melanocortins may play a role in the regulation of astrocyte morphology. Since changes in astrocyte morphology are observed in brain trauma35, occur during development48 and may also regulate interactions between neurons as well as neuron survival21, it is possible that these melanocortin induced changes may play an important role in astrocyte function. Phorbol esters were shown to induce significant morphological changes in these cells as well24. The cAMP induced morphological differentiation described here might however represent only a general nonspecific effect rather than a reflection on a real physiological process. It may be helpful to characterize the response of astrocytes to melanocortins in terms of biochemical markers, such as glutamine synthetase activity6,54, or changes in protein synthesis4 to determine if the morphological changes are being accompanied by changes in astrocyte function.

In addition, it was interesting to observe that both the
morphological changes, which we speculate to be cAMP mediated, and the proliferative effect could be induced by melanocortins in the 7-day-old cultures. The data presented clearly demonstrate that melanocortins are stimulating astrocyte growth under serum-free conditions but cannot exclude the possibility that they might exert a different effect in the presence of serum. The morphological assay, however, was conducted in the presence of 10% fetal calf serum and under these conditions, ACTH induced the morphological changes.

The influence of melanocortins on glial cells may be related to their more complex effects on the CNS. The temporal influence of melanocortins on immature, astrocyte proliferation might be intrinsically supportive to promote neuron survival and growth and synaptic plasticity during brain development. It has been demonstrated that the presence of glial cells is beneficial for the development and differentiation of neuronal cells in culture, as well as essential for neuronal migration.

In summary, this study shows that astrocytes in vitro respond to melanocortins. These findings are consistent with the possibility that astrocytes are targets for melanocortins in the CNS. Furthermore, two signal transduction mechanisms have been implicated in the regulation of the astrocyte specific responses. While it appears that proliferation is being mediated by a cyclic AMP independent mechanism that has still to be identified, the morphological changes appear to be regulated by a cyclic AMP dependent process.

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