α-MSH and $ACTH_{4-9}$ Analogue Org 2766 Induce a cAMP Increase in Cultured Rat Spinal Cord Cells

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The melanocortin α-MSH stimulates neuronal outgrowth of rat spinal cord slices (0.1–1 nM)[1] and of dissociated spinal cord cells (1–100 μM).[2] The $ACTH_{4-9}$ analogue Org 2766 has no effect on spinal cord slices and dissociated spinal cord cells. Both peptides, however, enhance the regenerative response in crushed sciatic nerves *in vivo*.[3] How these peptides exert their neurotrophic action is not known.[4,5] An MSH/ACTH receptor on neuronal cells has not yet been described. To study the mechanism of action of α-MSH, and find a clue as to why both peptides have different effects *in vitro*, we studied whether the cAMP second-messenger system is activated by these peptides.

Spinal cords of E15 Wistar rats were dissected and dissociated by enzymatic and mechanical means. Cells were cultured in poly-L-lysine-coated 24-well culture plates (235,000 cells/well), at 37°C in a humidified atmosphere under 5% CO_2. The culture medium consisted of DMEM supplemented with 5% v/v heat-inactivated horse serum, 4-mM glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin. After 48 h in culture, the cells were rinsed with DMEM (10 min), subsequently replaced by DMEM with 1-mM isobutylmethylxanthine (IBMX) (10 min). Thereafter the cells were treated for 10 min with α-MSH or Org 2766 (1 nM–100 μM) in the presence of 0.3-μM forskolin and 1-mM IBMX. Forskolin was added to increase the efficacy of the peptide effect.[6] Cells were fixed with 100% EtOH, and cAMP levels were measured with a radioimmunoassay (NEN-DuPont).

The cAMP activator forskolin showed a dose-dependent stimulation of cAMP production in cultured spinal cord cells (not shown). Treatment with α-MSH or Org 2766, in combination with 0.3-μM forskolin which augmented the cAMP production slightly, resulted in a cAMP increase with a maximum of 42% for α-MSH at 10 μM (FIGURE 1) and of 38% for Org 2766 at 1 μM (FIGURE 2). Both peptides showed a bell-shaped dose-response curve.

The present study shows that both peptides affect the production of cAMP in dissociated spinal cord cells with a characteristic bell-shaped curve. A similar curve was found for stimulation of outgrowth from intact spinal cord slices.[1] Others have shown that α-MSH and $ACTH_{4-10}$ regulate cAMP production in cultures of rat cortical neurons[6] and chick cortical neurons.[7]

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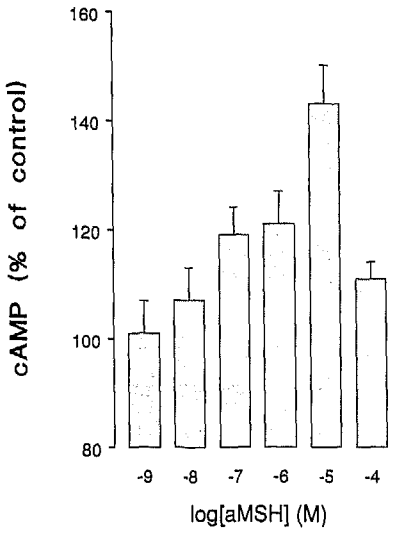

FIGURE 1. Dose-response curve of the effect of α-MSH on cAMP in cultured spinal cord cells. Each bar represents mean ± standard error of the mean (SEM) (n = 12).

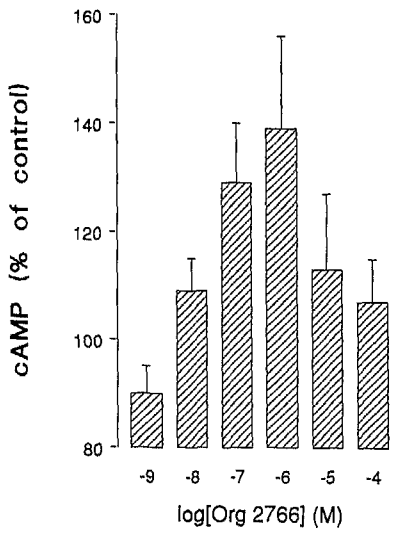

FIGURE 2. Dose-response curve of the effect of Org 2766 on cAMP in cultured spinal cord cells. Each bar represents mean ± SEM (n = 9).

Our results suggest that there are adenylate cyclase–coupled binding sites for α-MSH and Org 2766 on fetal spinal cord cells. The discrepancy between the cAMP induction (by both α-MSH and Org 2766) on one hand and outgrowth stimulation (only α-MSH) on the other suggests that stimulation of neurite outgrowth by α-MSH is not caused by activation of adenylate cyclase alone.

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