

Effects of glucagon on the growth of *Neurospora*

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Abstract

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Effects of Glucagon on the Growth of

Neurospora cc.

Adenosine 3'-5' monophosphate (cAMP) seems to be involved in the control of morphology and differentiation of Neurospora crassa; some morphological mutants of Neurospora show altered adenylate cyclase activity and cAMP levels (Scott 1976 Ann. Rev. Microbiol. 30: 85). However, it is still unclear whether cAMP is involved in the control of growth of Neurospora, as it is in other eukaryotes (Pastan and Johnson 1975 Ann. Rev. Biochem. 44: 493).

Membrane-bound adenylate cyclase activity isolated from the slime mutant has been shown to be activated by glucagon, and incubation of slime cells with this hormone led to an increase in glycogenolysis (Torres 1972 Proc. Natl. Acad. Sci., USA 69:2870). To determine if conditions that lead to an increase in endogenous cAMP levels also induce changes in growth rate, we studied the effects of adding glucagon to cultures of wild type (St. Lawrence 74A).

Glucagon (at concentrations up to $1 \times 10^{-5}M$) had no effect on the growth rate of young cultures, growing exponentially ($A_{450} = 0.2$) in Vogel's medium with 0.05% glucose. However, when the concentration of Vogel's medium was lowered to 1/20 of the normal amount, glucagon above $5 \times 10^{-6}M$ had a paramorphogenetic effect; after 30-60 min, hyphae aggregated and formed small balls of mycelium. In control cultures lacking glucagon, growth was normal at least up to A_{450} of 0.6. Attempts to find which component(s) of Vogel's medium prevented the paramorphogenetic effect of glucagon suggested that the ionic strength (and/or the buffering capacity) of the medium must be reduced to demonstrate the effect of glucagon. An analogous morphogenetic effect was found by Mishra *Naturwissenschaften* 1976 63: 485) upon the addition of cAMP to liquid cultures. In the presence of $9 \times 10^{-6}M$ glucagon, both RNA and protein accumulation was inhibited by 30-40%, while $7 \times 10^{-5}M$ glucagon completely blocked accumulation of these macromolecules. {Accumulation of RNA and protein was monitored by incorporation of radioactive precursors (3H -uridine and ^{14}C -leucine).} Glucagon ($9 \times 10^{-6}M$) had no effect on the rate of protein degradation, which was negligible in the presence or absence of glucagon (Martegani and Alberghina 1979 *J. Biol. Chem.* 254: 7047).

Glucagon also affected glycogen metabolism. In early exponential growth of slime cells, glycogen level (measured according to Stewart (1975 *Methods in Cell Biol.* 12: 111)) was ca. $55\mu/A_{450}$ unit of culture. Glucagon addition ($9 \times 10^{-6}M$) inhibited glycogen accumulation by about 50% in 90-120 min.

Therefore adding glucagon to Neurospora wild type cultures causes: a) morphological changes similar to those caused by addition of cAMP, b) inhibition of glycogen accumulation, and c) inhibition of growth. It seems likely that these effects are related to an increase of the endogenous cAMP level.

In considering these results, it is interesting to note that a protein hormone is produced during the sexual cycle of Neurospora, which induces both the aggregation of hyphae for the formation of protoperithecia and the synthesis of tyrosinase, which is also induced by cAMP (Scott 1976 Ann. Rev. Microbiol. 30: 85). It has recently been proposed (Trevillian and Pall 1979 *J. Bacteriol.* 138: 397) that elevated cAMP levels, induced in Neurospora by depolarization of plasma membrane, stimulate cell wall biosynthesis, suggesting a biochemical basis for the morphogenetic effect induced by glucagon. - - - Centro del C.N.R. per la Biologia Cellulare e Molecolare delle Piante, Istituto di Scienze Botaniche, e Cattedra di Biochimica Comparata, Università di Milano, Italia.