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CHANGES IN SPECIFIC ACTIVITIES OF 3′,5′-CYCLIC ADENOSINE MONOPHOSPHATE PHOSPHODIESTERASE IN CHICK EMBRYO CELLS GROWN UNDER DIFFERENT CONDITIONS

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Summary. — The relationships between the density of chick embryo cells in stationary cultures and their specific 3′,5′-cAMP phosphodiesterase (PDE) activities and calmodulin (CaM) contents were investigated. An indirect relationship between them was found. The specific CaM-PDE activities were the highest in the starting logarithmic phase of growth and the lowest in grown (72 hr old) monolayer cultures. Changes in the specific activities induced by two serum concentrations were also related to the cell culture density. The results suggested that the cell density or any metabolic change due to it is the primary factor regulating the level of specific PDE activities in chick embryo cells.

Key words: chick embryo cells; 3′,5′-cyclic adenosine monophosphate phosphodiesterase; calmodulin

Introduction

Križanová *et al.* (1977) showed that interaction of influenza virus with the cell membrane results in the inhibition of calmodulin- (CaM) stimulated 3′,5′-cAMP phosphodiesterase (CaM-PDE) activity. CaM is necessary for the first step of virus infection of the cell (Križanová *et al.*, 1982).

In an attempt at affecting the virus infection by changes in the specific activities of PDE or CaM of susceptible cells, we studied these activities in chick embryo cells (CEC) grown under different conditions in stationary cultures.

Materials and Methods

CEC were obtained by trypsinization of 11 days old chick embryos (Dulbecco and Vogt, 1954). They were seeded into 300-ml Roux (3×10^7 cells) or Müller (5×10^6 cells) bottles in 30 or 5 ml, respectively of basal Eagle s medium (BEM) supplemented with 10% inactivated calf serum (ICS). The monolayers became confluent usually after 72 hr. At intervals of 30, 45—48 and 70—72 hr the cultures were twice washed at 0 °C with 0.14 mol/l NaCl buffered with 10 mmol/l Tris-HCl buffer at pH 7.5, then with 0.25 mol/l sucrose in 10 mmol/l Tris-HCl buffer pH 7.5 and frozen in a dry ice — ethanol mixture. After scraping off into a small volume (the contents from 5 bottles into 2 ml of 20 mmol/l Tris-HCl buffer pH 7.5 containing 1 mmol/l dithiothreitol

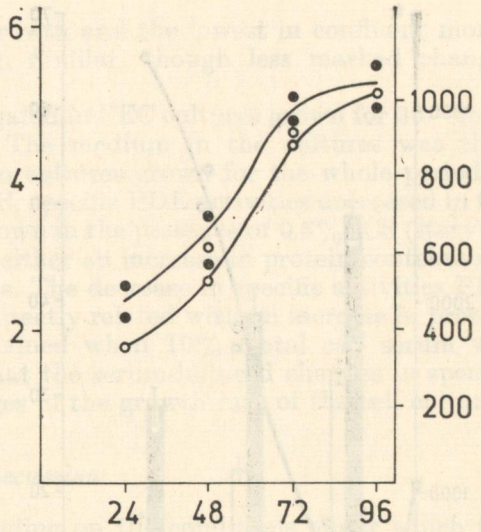

Fig. 1.
Growth curve of CEC in monolayer cultures
Abscissa: time of cultivation in hr; left ordinate: cell counts (in millions); right ordinate: μg protein
○ — μg protein per Müller bottle
● — No. of cells released by trypsin from one Müller bottle
Mean values from three determinations

and sonication for 2 min at 15 kHz, the homogenates were centrifuged for 30 min at 4000 rev/min (2000 × g). The supernatant was used for protein and PDE activity assay. CaM was assayed in supernatants (extracts) heated for 3 min at 100 °C and clarified at 4000 rev/min for 30 min.

Cell growth was estimated by determining the counts of cells released by trypsinization according to Bulinski and Borisy (1980) or the protein contents in cell monolayers twice washed with phosphate buffered saline (PBS; Dulbecco and Vogt, 1954) and dissolved in 1 (Müller bottle) or 2 (Roux bottle) ml of 1 mol/l NaOH. In the former case, cells were released from Müller bottles with a 0.25% trypsin solution. The action of trypsin was stopped after 5 min by adding 2 mg/ml bovine serum albumiun and 0.1 mg/ml soybean trypsin inhibitor. The cells were centrifuged off, resuspended in growth medium, stained with 0.2% erythrosin and counted. Proteins were determined according to Lowry *et al.* (1951).

PDE activity was assayed in 0.3—0.5 mg of protein homogenates by the method of Dedman and Means (1977) as modified by Križanová (1979) in the absence (total) or presence of 0.5 mmol/l ethylene glycol-bis-(2-amino ethyl ether)-N, N-tetraacetic acid (EGTA). With each sample, a parallel blank without 3′,5′-cAMP was assayed; its value was subtracted from the samples value. CaM-dependent PDE activity was calculated from the difference in the activities determined in the absence and presence of EGTA. Specific activities were expressed in nmoles 3′ 5′-cAMP hydrolysed in 30 min at 30 °C per mg protein in the supernatant.

The CaM levels were calculated from the degree of stimulation of the isolated CaM-dependent PDE by a diluted heated extract and expressed as per cent of maximal stimulation per 10 μg protein homogenate. Maximal stimulation was determined with the use of purified CaM (Križanová *et al.*, 1979). CaM-dependent PDE and pure CaM were prepared from chick embryos by DEAE-cellulose column chromatography (Križanová *et al.*, 1979).

Chemicals. Dithiothreitol was purchased from Sigma; 3′,5′-cAMP, imidazole and adenosine deaminase from Calbiochem, Crotalus atrox venom from Koch-Light; EGTA from Fluka; and ICS from Bioveta Ivanovice na Hané.

Results

The growth of CEC in Müller bottles as determined by protein contents and cell counts is illustrated in Fig. 1. The growth of cells that became attached to the glass was rapid during the first 65—70 hr after seeding. It slowed down when the cells approached confluency (72 hr).

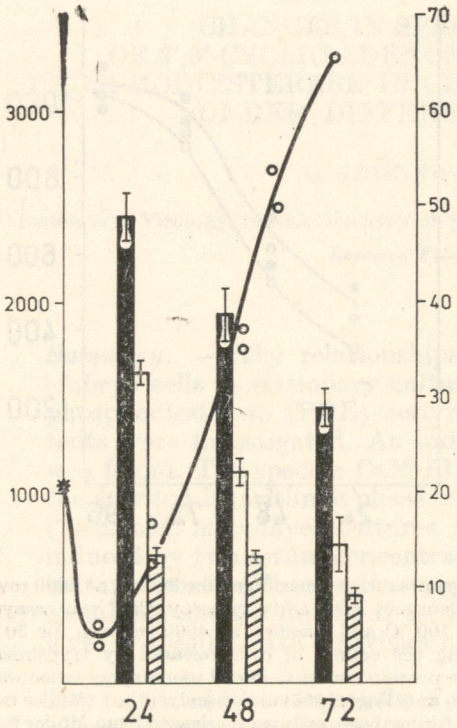

Fig. 2.

Specific PDE activities during growth of CEC in stationary cultures
Abscissa: time of cultivation in hr; left ordinate: μg protein; right ordinate: specific PDE activity or CaM contents.
Specific activity of total PDE (black column).
Specific activity of CaM-stimulated PDE (empty column).
CaM contents (dashed column).
Growth curve of CEC in a Roux bottle as determined by protein contents (○———○).
* Proteins in the starting cell seed (asterisk)
Mean values from 5 experiments

The relationship between specific PDE activities and cell growth was investigated in Roux bottle cultures. The results (Fig. 2) revealed an indirect relationship between cell growth and their specific CaM-PDE activities which are responsible also for changes in total PDE. Specific activities were

Table 1. The effects of ICS concentration in nutrition medium on cell growth and specific PDE activities in stationary CEC cultures

Type of culture		mg protein per Roux bottle	Specific PDE activity		CaM contents
			Total	CaM-stimulated	
A	0.5% ICS	1.98	38 ± 2.5	24 ± 2	12.6 ± 0,8
	10% ICS	3.56	31.3 ± 1.7	18.6 ± 2.6	10
B	0.5 ICS	2.0	35	15	
	10% ICS	3.7	25	12	
C	0.5 ICS	1.66	41	29	15
	10% ICS	4.35	34	20	11

A — 48-hr-old cultures incubated for another 18 hr in BEM containing 0.5 or 10% ICS.
B — 48-hr-old cultures starved for 18 hr in BEM containing 0.5% ICS and subsequently incubated for another 24 hr in BEM containing either 0.5 or 10% ICS.
C — 30-hr-old cultures incubated for another 42 hr in BEM containing either 0.5 or 10% ICS.

the highest in the early phase of growth and the lowest in confluent monolayers at 70—72 hr after seeding. Similar, though less marked changes occurred in the level of CaM.

The effects of serum were investigated in CEC cultures grown for 30—48 hr in medium containing 10% ICS. The medium in the cultures was then changed as indicated in Table 1. In cultures grown for the whole period of incubation in medium with 10% ICS, specific PDE activities decreased in the course of cell growth. In cultures grown in the presence of 0.5% ICS (starving cultures) for 18 hr there occurred neither an increase in protein contents nor a decrease in specific PDE activities. The decrease in specific activities PDE in the presence of 10% ICS was indirectly related with an increase in protein contents. Similar results were obtained when 10% foetal calf serum was used instead of ICS. We assume that the serum-induced changes in specific PDE activitiews were due to changes in the growth rate of the cell cultures.

Discussion

PDE activity may change depending on the conditions under which the cells are grown. Russel and Pastan (1973) and others have shown that it is induced by an increase in the level of 3′,5′-cAMP in the cell. Strada and Thompson (1978) suggested that different forms PDE occur in different cells (e.g., BHK and Balb and 3T3) and that cultivation conditions may result in changes in the form, amount and substrate specificity of phosphodiesterase activities. In CEC, we observed an indirect relationship between cell density in a stationary culture and the specific CaM-PDE activities. Dutta-Gupta et al. (1978) found that specific activities of low Km 3′,5′-cAMP, high Km 3′,5′-cAMP and 3′,5′-cGMP PDE in cultures of human WI-38 fibroblasts increase during growth of the cells in culture from low to medium density and then decrease with a further increase in the cell density. Although not great, these changes were significant in numerous experiments. It is possible that in our experiments we dealt with this second phase of cell growth only. Changes in PDE activities in CEC can be hardly compared with those described by Pledger *et al.* (1976, 1979) in BHK cells of hamster origin. Two different animal species are involved and the localization and forms of PDE in the cells are different. BHK cells contain no particle-bound PDE (Strada and Thompson, 1978). In parallel pilot experiments (unpublished) we found no CaM-PDE activity in BHK cells and the specific activity of PDE was in BHK cells lower than in similarly treated CEC.

As distinct from 0.5% ICS, the addition of 10% serum to the growth medium supported cell growth but reduced the specific PDE activities. These results suggest that the primary factor regulating the level of specific PDE activities in CEC is the cell density or some factor closely connected with it.

The susceptibility to virus of cells showing different specific PDE activities could not be tested by the plaque method since the latter requires confluent cell monolayers.

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