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CHANGES IN ECTO-5'-NUSLEOTIDASE ACTIVITY DURING EARLY STAGES OF VESICULAR STOMATITIS VIRUS INFECTION. EFFECTS OF CONCANAVALIN A

J. L. DAVEZE, J. FRAPPA, Y. BEAUDRY, R. DELOINCE, R. FONTANGES

Division de Microbiologie du Centre de Recherches du Service de Santé des Armées, 69272 Lyon Cedex 1 — France

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Summary. — Study of ecto-5'-nucleotidase was performed in cultured epithelial cells, infected with vesicular stomatitis virus (VSV). Changes in the enzymatic activity were measured in terms of $TCID_{50}$ and exposure time. The effects of an enzyme inhibitor, Con A, were also investigated during the interaction between virus and cells.

Key words: vesicular stomatitis virus; foetal calf oral epithelial cells; 5'-nucleotidase; Concanavalin A

Introduction

Ecto-5'-nucleotidase in animal cells is almost exclusively located on external surface of the plasma membrane. Thus it is accessible to the substrate 5'-AMP to which it exhibits a complete affinity (Depierre *et al.*, 1975). Its kinetics can be determined on whole cells using Con A as a specific inhibitor of this enzyme (Stephanovic *et al.*, 1975). Carraway *et al.* (1976) suggested that ecto-5'-nucleotidase could be useful for checking the changes within plasma membranes. Virus induced variations in enzyme levels have been studied previously, particularly in early stages of virus-cell interactions (Poste *et al.*, 1972; Di Pietro *et al.*, 1977).

In this report we followed the early effects of VSV infection on the level of 5'-nucleotidase using Con A to determine the specificity of the enzyme activity.

Materials and Methods

Cultured cells. Cultures of pure foetal calf oral epithelial cells have been prepared as described (Frappa *et al.*, 1971). They were grown in Eagle's Basal Medium with Earle's salts, supplemented with 10% foetal calf serum, 200.000 U/l penicillin and 50 mg/l streptomycin.

Virus. Vesicular stomatitis virus, strain IFFA (1), batch 21277, was maintained on foetal calf oral cells and kept frozen at —70 °C until use. Titration asssays were carried out in microplates. In addition, virus supension from the same batch, heat-inactivated for 30 min at 56 °C, exhibiting no cytopathic effect on cell monolayers, was also used.

Experimental design. Confluent cell monolayers grown in Roux bottles were washed three times with 20 ml phosphate buffered saline (PBS), dissociated by gentle agitation at 37 °C in 10 ml of PBS supplemented with 0.5% EDTA. The harvested cells were pooled, washed

twice by centrifugation in PBS for 10 min at 1500 × g and finally resuspended in PBS containing 5 mmol/l Mg^{2+}. Cell counts were performed on a Nageotte haemocytometer. Viability was determined by exclusion with Trypan blue. Cells were centrifuged again, resuspended in serum-free medium at 37 °C and separated into two lots: one was exposed to the native virus, the other one to the heat-inactivated one. Control tests were performed with serum-free medium. One ml samples were immersed in PBS supplemented with 5% calf serum kept at 0 °C in order to block virus effects. Cells were washed twice and resuspended in a suitable incubation medium (Tris-HCl 80 mmol/l, NaCl 80 mmol/l, KCl 40 mmol/l, $MgCl_2$ 5 mmol/l; pH 7.4).

Various virus concentrations were utilized (10, 250, 500 and 1000 $TCID_{50}$ per cell). Exposure times varied from 10 to 15, 30 and 60 min. A 500 $TCID_{50}$ per cell virus suspension was maintained in contact with the cells for 15 min in culture medium supplemented with Con A. The lectin was used in concentrations of 0.07 mmol/l or 0.2 mmol/l.

Protein measurements. The cells were precipitated with 5% TCA, centrifuged at 9000 × g for 5 min and the pellet was dispersed in 0.1 mol/l NaOH. Proteins were determined according to Lowry *et al.* (1951).

5'-nucleotidase activity. 0.3 to 0.5 × 10^6 cells suspended in the incubation medium (final volume 0.8 ml) were immersed in a 37 °C waterbath. The reaction was initiated by adding 5'AMP (pH 7.4) and stopped after 30 min with 75 µl 50% cold TCA. The cells were centrifuged at 9000 × g. Mineral phosphate released in the supernatant was evaluated according to Sumner (1944), or with malachite green when very low amounts of mineral phosphate were involved (Muszbek *et al.*, 1977). Each result was compared to a control test in which TCA was added prior to 5'-AMP.

Previous studies have shown (Daveze *et al.*, 1978) that maximal activity was achieved from 3 mmol/l 5'-AMP onwards, and remained constant in standard conditions regardless of Mg^{2+} concentration (0 to 10 mmol/l). Two enzymatic activities were involved in this measurement: ecto-5'-nucleotidase and non-specific phosphomonoesterases from the cell membrane and lysed cells. Using p-nitrophenylphosphate as a substrate, according to Depierre and Karnowsky (1974), the percentage of 5'-AMP hydrolysed by non-specific phosphomonoesterases approximated 3%.

Results

Maximum basic activity of ecto-5'-nucleotidase in foetal calf oral cells in the 20th and the 30th passages was determined (6.5 ± 0.5 µM of mineral phosphate released per hr per mg protein). Con A-induced inhibition of the enzyme activity has reached 80% at concentration of 0.2 mmol/l; it decreased to 50% at 0.07 mmol/l concentration (Daveze *et al.*, 1978).

Native VSV-induced transient changes in the activity of ecto-5'--nucleotidase from 10 to 60 min after exposure. Their extent varied according to different virus concentrations (Fig. 1-I). By 10 min after exposure to the native virus the enzyme activity was decreased, but at 15 and 30 min it was enhanced. Later on by 60 min the enzyme activity has decreased again. The changes of enzyme activity induced with the inactivated VSV were similar (Fig. 1-II). However, no decrease in the enzyme activity occurred at 60 min after exposure.

After exposure of the cells to 1000 $TCID_{50}$ VSV per cell, the changes in the ecto-5'-nucleotidase activity were minimal regardless whether native or inactivated virus had been used. A slight increase in the enzyme activity was followed by a minimum elevated level throughout the observation period.

The enhanced activity of ecto-5'-nucleotidase 15 min after the exposure to 500 $TCID_{50}$ VSV per cell could be reversed in the presence of Con A. An

effective inhibition of the enzyme was achieved at the concentration of 0.2 mmol/l Con A, regardless whether cells were exposed to the native or to the inactivated virus.

Discussion

In cultured diploid oral cells, the activity of ecto-5'-nucleotidase is similar to that of transformed cells (Stephanovic *et al.*, 1975; Carraway *et al.*, 1976; Di Pietro *et al.*, 1977). This activity does not seem to depend upon Mg^{2+}

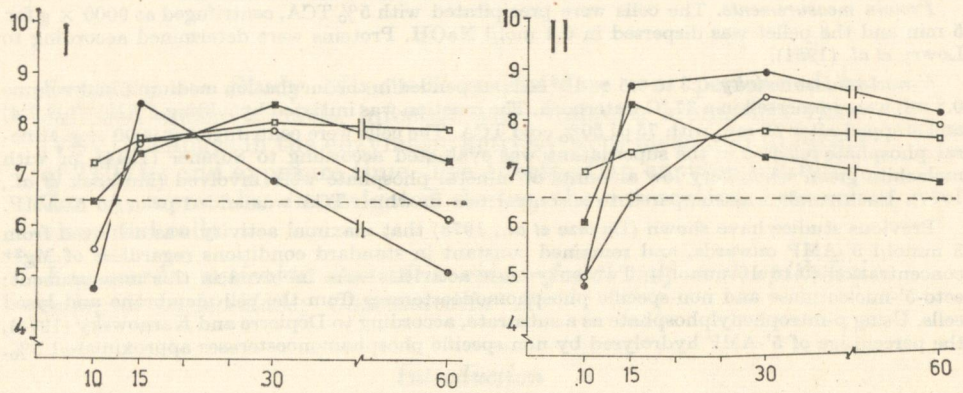

Fig. 1.

Changes in 5'-nucleotidase activity in the presence of native or inactivated virus
Control — interrupted line
●—● 10 ID_{50}/cell; ○—○ 250 ID_{50}/cell; ■—■ 500 ID_{50}/cell; □—□ 1000 ID_{50}/cell
I — native virus; II — inactivated virus
Abscissa: exposure time (min); ordinate: 5'-nucleotidase activity (μM Pi released per hr and per mg protein)

concentration in the medium (Daveze *et al.*, 1978). Regardless to cell type, the affinity of this enzyme for 5'-AMP is always close to Km = 0.14 mmol/l, provided cells remain intact (unpublished results). The penetration of attached VSV begins within a few minutes after the initial exposure and reaches a maximal level after about one hour (Wiktor and Koprowski, 1974). The results of ecto-5'-nucleotidase activity measurements suggest that a transient change in the enzymatic activity takes place during the interaction between the cell membrane and the native or inactivated virus. These changes are directly correlated with the exposure time. Lower dilutions induce maximal effects. With such dilutions, the effects yielded by the native virus were different from those obtained with the inactivated form (Fig. 1) for long exposure periods at present, we do not know the reason of these differences.

Experiments were carried out infecting oral foetal cells with Mahoney strain I of poliovirus. The results were similar, although this virus exerts no cytopathic effect on those cells after 72 hr exposure (unpublished results).

Table 1. Reversion of ecto-5'-nucleotidase stimulation by VSV in the presence of Con A

Period of experiment	No Con A	Con A concentration of 0.07 mmol/l			Con A concentration of 0.2 mmol/l		
		C	IV	NV	C	IV	NV
Prior absorption	6.5**	3.4 (52%)**	4.6 (55%)	4.7 (65%)	2.7 (42%)	2.8 (32%)	3.5 (49%)
During absorption*	8.4	4.0 (62%)	5.0 (59%)	6.4 (89%)	1.4 (22%)	1.4 (16%)	1.2 (17%)
After absorption	7.2	3.4 (55%)	3.6 (42%)	4.8 (67%)	3.1 (48%)	3.1 (36%)	2.4 (33%)

* 500 $TCID_{50}$ of VSV for 15 min.
** Activity in terms of μM Pi released per hr and mg protein (% of the value in the absence of Con A).
C = control; IV = inactivated virus; NV = native virus.

Thus, the enzymatic response does not seem to depend upon the virus species. After 15 min exposure with 500 ID_{50} per cell, at concentration of 0.2 mmol/l, Con A provides an inhibition similar to that observed in controls, but not when the native virus was added at concentration of 0.07 mmol/l Con A. In the last case, we think that Con A might be fixed to Con A receptors of the virus (Becht *et al.*, 1972; Calafat *et al.*, 1972) and so its level in the medium might decrease. VSV did not seem to interfere with Con A (Table 1). Likewise, the receptor blockade specific for coxsackie B viruses did not alter the Na-K dependent ATPase (Crowell, 1976).

The ATPase exerted an inhibiting influence on the cytopathic effect of VSV. The inhibition was related directly to the concentration of ATPase, and inversely to the infectious dose of the virus. The effects of a virus-induced infection on ecto-5'-nucleotidase activity are complex and the findings of this study need further investigations.

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