Vol. 86, No. 4, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1979 Pages 1102-1109

THERMODEPENDENCE OF ADENYLATE CYCLASE IN KB CELLS INFECTED BY SENDAI VIRUS : INFLUENCE OF CA^{++}IONS

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Received January 11,1979

SUMMARY

Temperature affects adenylate cyclase activity. There is a break at approximately 20°C in Arrhénius plots obtained with control as with inoculated cells. A preincubation of cells with 10 mM Ca Cl_2 shifs this break up to 30°C. Below the temperature of transition adenylate cyclase of cells inoculated with enough virus to get cell-fusion at 37°C, is little dependent on temperature.

INTRODUCTION

An arrangement of phospholipids in a bilayer with globular proteins immersed in the lipid phase is now admitted for the structure of plasma membrane (1). Membrane fusion is a physiological process involved in a variety of life phenomena (2). Cell fusion induced by Sendaï virus has been studied in detail (3,4). The first step, at a low temperature, is an adsorption of virus on a mucoprotein of the host cell, then the viral enveloppe fuses with and is integrated into the plasma cell membrane. Cell fusion is initiated by incubation at 37°C. Adenylate cyclase is a membrane-bound and multiregulated enzym system that is influenced by the structure of the membrane and the physical properties of the lipids. The inflections in the Arrhenius plots of the activity of membrane-bound

0006-291X/79/041102-08$01.00/0

enzymes have been attributed to lipid phase transitions in the membrane (5,6).

We have recently shown (7) an adenylate cyclase activation in KB cells infected with a little dose of Sendaï virus. In the present paper, we describe the effects of temperature on adenylate cyclase activity of KB cells infected by Sendaï virus. Two conditions of inoculation have been chosen: a little dose of virus (10^{-2}EID 50/cell) so that cells do not show cytopathic effects and a large dose (200 EID 50/cell) to get generalized cell fusion. We have performed a study first, on the influence of viral material on lipid transitions correlated to adenylate cyclase activity and secondly, on the influence of Ca^{++} ions implicated in the fusion processes (8) and known to induce phase changes in phosphatidylserine vesicles (9).

MATERIAL AND METHODS

KB cells were grown in Eagle's minimal essential medium supplemented with 10 % calf serum as precedently described (7,10) Sendaï virus was propagated by periodic inoculations of 10-day-old-embryonated eggs. Fifty per cent end point (EID_{50}) was calculated by the method of Reed and Muench (11) on log-probit paper (12). The virus titer was $10^{9,5}$ EID50/ml. Cells were inoculated then harvested and broken as precedently described (7,10).

Adenylate cyclase activity was measured in 1000g pellet as previously reported and discussed (10). The assay medium included 2 mM (α^{32}P)-ATP 1µ Curie, 1mg/ml creatine phosphate, 1mg/ml serum albumine bovine, 10 mM $MgCl_2$, 10 mM theophylline, 25 mM Tris-HCl $_{p}$H = 7,6. The reaction was initiated by addition of enzyme (approximately 400µg protein) at the indicated temperature, incubation was carried out for 10 minutes at the indicated temperature and the reaction was stopped by immersion in boiling water for 3 minutes and addition of "stop solution" containing 20 mM ATP, and 6 mM (8^{3}H)-cyclic AMP as chromatographic tracer. Cyclic nucleotide was isolated and measured as described by White and Zenzer (13). In all the cases, activities were linear with respect to protein concentration up to 1 mg and to time of incubation up to 20 minutes.

The slopes of Arrhenius plots and so the existence and location of break points were determined by the least squares method.

RESULTS AND DISCUSSION

Temperature affects adenylate cyclase activity as shown in Figure 1. Arrhenius plots obtained with control cells as with inoculated cells present of discontinuity

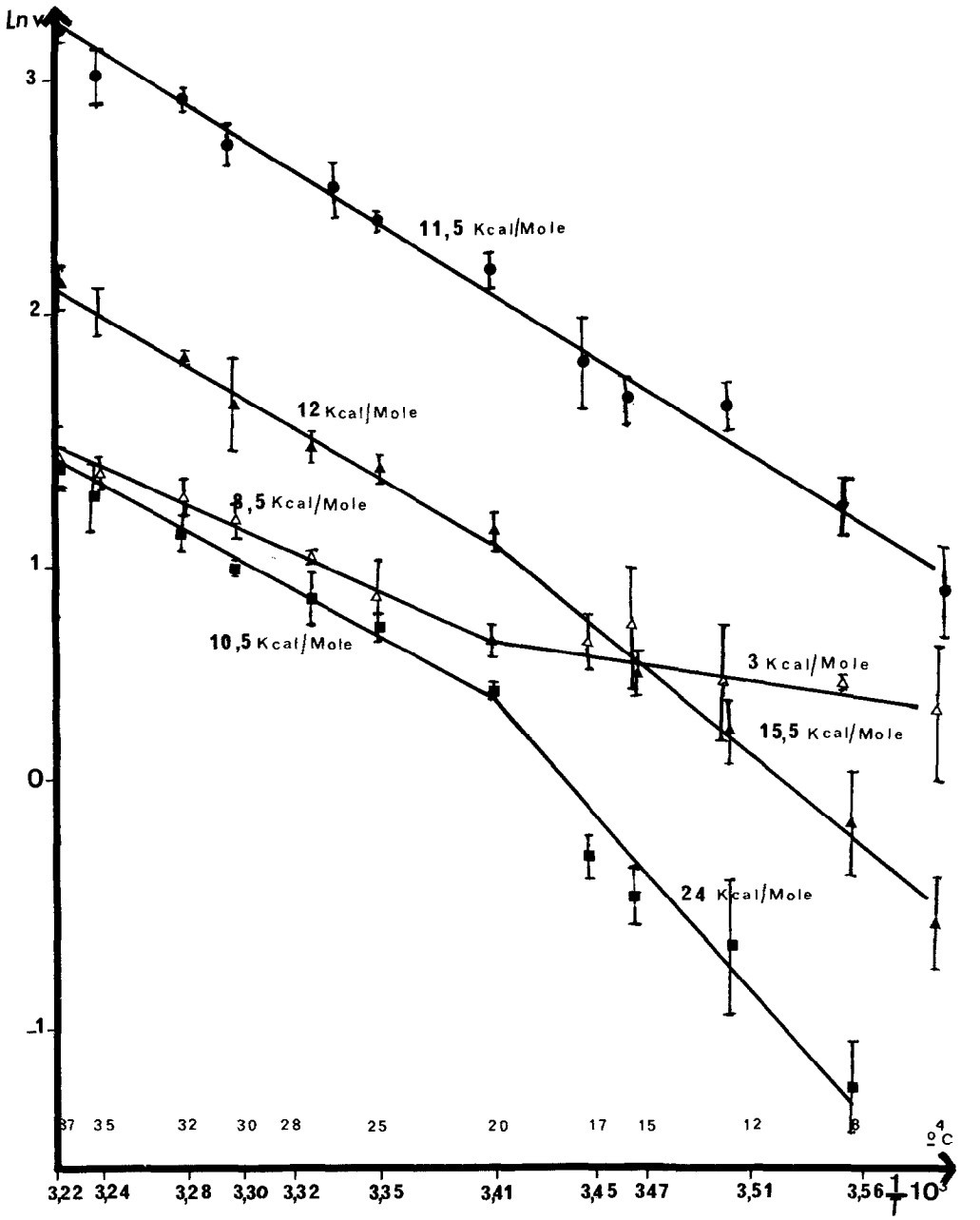

Fig. 1 - Thermodependence of 1000 g pellet adenylate cyclase activity : Arrhenius plots. Virus inoculation, one hour incubation and adenylate cyclase assay were carried out at the indicated temperature. Three flasks were pooled for each assay which is carried out in duplicate. Results are mean of three experiments. (v : pMoles cAMP/m/mg prot.) ■ Control cells. ▲ Cells inoculated with 10^{-2} EID_{50}/cell. △ Cells inoculated with 200 EID_{50}/cell. ● Cells with 10 mM Na F.

at approximately 20°C. A supposed transition in the lipid phase may influence the enzyme activity, the lipids may physically interact between the catalytic site and some regulator sites perhaps some effectors usually coupled with the enzyme.

Above the break point, adenylate cyclase activity of cells inoculated with a little dose of virus is higher than that of control cells but a large dose of virus does not significantly change the activity as precedently shown (7). The activation energies are on the same range. Below the break point, a large dose of virus increases more strongly adenylate cyclase than a little dose and the activation energy is very low. The activity seems to be very little influenced by temperature. The plot of activity stimulated by 10 mM NaF is linear, it does not present a discontinuity, the activity is insensitive to any phase separation occuring in the bilayer.

These results suggest that the adenylate cyclase of the membranes in which virus particles are integrated remains functionnaly regulated or coupled in a similar manner to that of the membrane of control cells because they present the same break point, but that below this break point, the presence of a great number of virus particles lead to a"freezing" of the structure of the lipid bilayer . In the opposite, the presence of NaF makes the enzyme uncoupled, perhaps by a conformationnal change of structure.

The membrane-membrane and cell fusion processes have been shown (8) absolutly dependent on the rise of intracellular Ca^{++} ions. A preincubation of cells for 10 minutes with 10 mM $CaCl_2$ just before virus inoculation increases cells fusion which is achieved over 12 hours instead of 24 hours in our culture conditions. Calcium ions were found to be inhibitory to most of the adenylate cyclase (14). The addition of $CaCl_2$ directly in the assay medium in the standard conditions at 37°C. decreases stongly adenylate cyclase activity of control KB cells as shown in Figure 2, but in the case of cells inoculated with a large dose of virus, the activities are not significantly

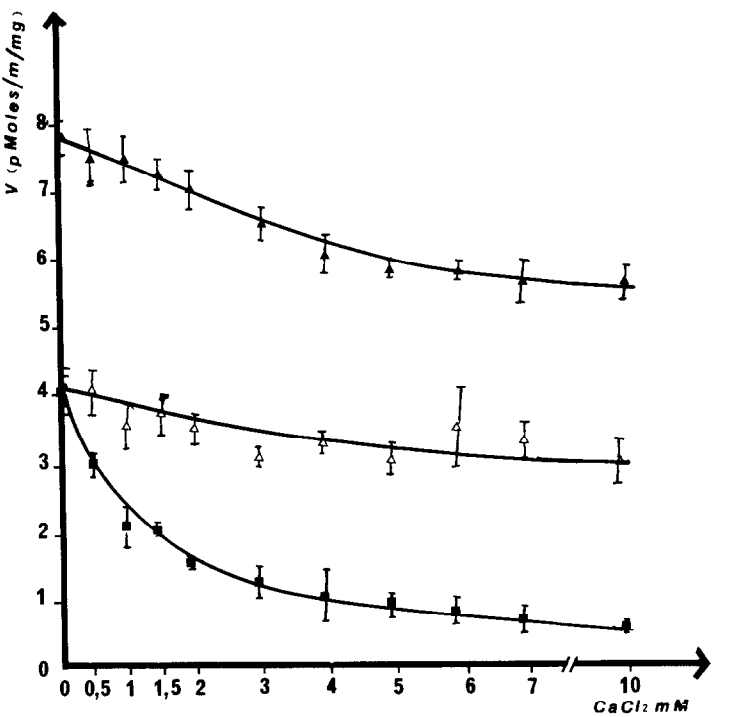

Fig. 2 - Influence of Ca^{++} ions on 1000 g pellet adenylate cyclase activity. Ca^{++} ions were added in the assay medium and activities were assaied in duplicate in the standard conditions at 37°C. Results are the mean values of three experiments. ■ Control cells. ▲ Cells inoculated with $10^{-2}EID_{50}$/cell. △ Cells inoculated with $200EID_{50}$/cell.

changed by the addition of $CaCl_2$ from 0 to 10 mM. In the case of cells inoculated with a little dose of virus, inhibition occurs but is slighter than in the case of control cells.

To better characterize the relation between the catalytic unit of adenylate cyclase and the physical state of the membrane we have studied the influence of temperature on adenylate cyclase activity of KB cells preincubated for 10 minutes with 10 mM $CaCl_2$ just before virus inoculation. Control cells are preincubated in the same conditions. Results are show on Figure 3. Calcium ions change the temperature of the break point on Arrhenius plot in control cells as in inoculated cells, temperature of transition is shifted up to 30° C. by the preincubation but Ca^{++} ions have no influence on NaF stimulated activity. The activation

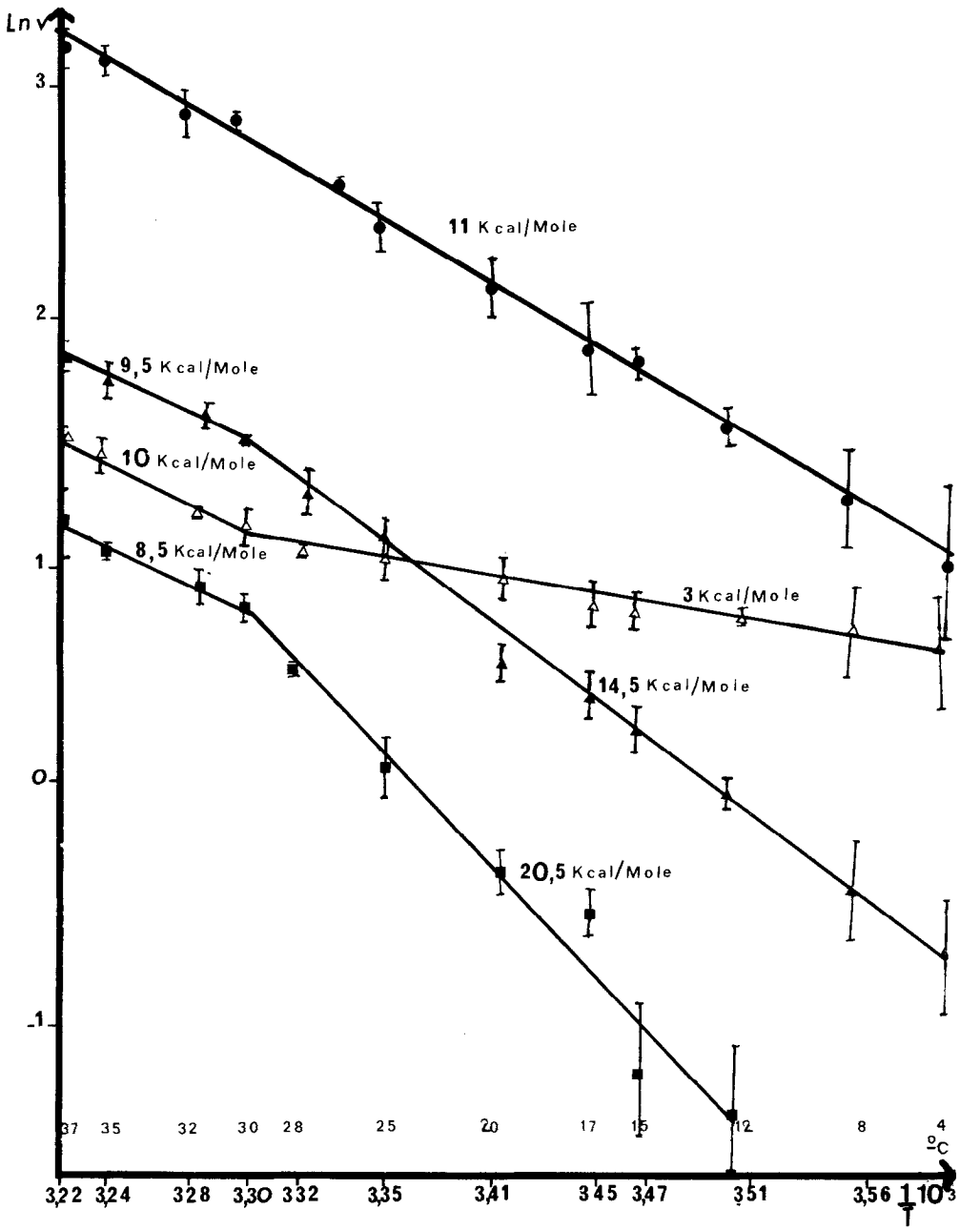

Fig. 3 - Influence of a preincubation of the cells with 10 mM Ca^{++} ions on the thermodependence of 1000 g pellet adenylate cyclase activity : Arrhenius plots. Virus inoculation, one hour incubation and adenylate cyclase assay were carried out at the indicated temperature. Three flasks were pooled for each assay which is carried out in duplicate. Results are mean of three experiments. (v = pMoles cAMP/m/mg prot.)
■ Control cells. ▲ Cells inoculated with $10^{-2}EID_{50}$/cell.
△ Cells inoculated with $200EID_{50}$/cell. ● Cells with 10 mM Na F.

energies are not changed by the preincubation with Ca^{++} ions except in control cells, the energy of which is significantly decreased below the break point.

The Ca^{++} ions were shown to promote domain separation in the phospholipid bilayer (9). In this work, we show that the presence of Ca^{++} ions change the thermodependence of the fatty acids surrounding the adenylate cyclase in a similar manner withand without the presence of virus particles but these viral particles block the inhibitory effects of Ca^{++} ions on the enzyme activity observed in control cells.

Factors changing cell membrane structure or delaying cell membrane movements influence cell fusion. In this way, Ca^{++} ions, responsable for creating an intermediate unstable state (9), active cell fusion and saccharides which inhibit cell membrane movements, block the fusion (15). More extensive work is needed to know if the concomitant modifications of adenylate cyclase activity are a consequence of these events or play a role in a stage of cell fusion. In this regard, a possible point of cyclic AMP action might be on the microtubular organization which determines both cell morphology and membrane transport (16) and the integrity of which is influenced by cyclic nucleotides (17,18). Indeed, recently, depolymerization of microtubules has been related to the induction of cell fusion (19, 20).

This work was supported by the U.E.R. Biologie Humaine-Université Claude Bernard - LYON - FRANCE.

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