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Elevation of the Intracellular Levels of Cyclic Adenosine Monophosphate during in vitro Infection of Transmissible Gastroenteritis Virus

Brief Report

By

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Cyclic 3′, 5′ adenosine monophosphate (cAMP) is believed to carry out a variety of intracellular functions as a second messenger when membrane-bound adenyl cyclase is activated by specific informational molecules such as hormones (1). In addition to its role as a second messenger, cAMP is also shown to be elevated or decreased intracellularly under some rather unexplained situations (2). Elevated levels of cAMP have been suggested as the immediate cause of the gastroenteric symptoms of cholera (3). Transmissible gastroenteritis virus (TGE) is a corona virus and is the etiological agent of a highly infective disease of newborn swine. Like cholera, the manifestations of this disease include vomition and a characteristic diarrhea (4).

In this report the effect of both TGE infection on cAMP levels of primary porcine kidney (PPK) cells and the effect of exogenously added cAMP on TGE specific RNA synthesis have been described.

The confluent monolayer of PPK cells (in 160 cm^2 Blake bottles) under standardized conditions were infected with cell-adapted Purdue strain of TGE virus at a multiplicity of 0.1 p.f.u. per cell and incubated at 37° C. At various intervals, the cells were harvested after washing twice and were extracted with 4—5 ml ice cold 5 per cent perchloric acid (PCA). The PCA-treated cells were then homogenized in a Ten-Broeck type glass homogenizer. The acid soluble fraction was neutralized to pH 7 with saturated potassium hydroxide solution and the salt-free supernatant was lyophilized. All operations were carried at 0—4° C. The cAMP content of the reconstituted residue was determined by the method of Gilman (5). The standard error of this assay as carried out in this laboratory is ± 5 per cent and the phosphodiesterase sensitive recovery of cyclic AMP is determined to be 90 per cent.

The results of cAMP determination as a function of time, post infection, are shown in Figure 1. The sharp rise of cAMP after 30 minutes in the infected cultures was not observed either in uninfected cells or cells treated with TGE virus inactivated with 0.1 per cent β-propiolactone. However, the increase in cAMP level is not proportional to viral input and at m.o.i. of 10 p.f.u., the initial rise of cAMP at 30 minutes post infection is 50 per cent lower than the corresponding value at m.o.i. = 0.1, but this elevated intracellular level persists till 12 hours post infection. Such a rise of intracellular cAMP suggested its function as being either a membrane response to the viral infection or its involvement in a possible derepression of cell genome required for viral expression.

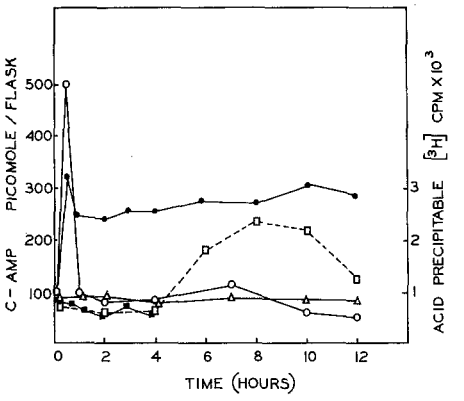

Fig. 1. Kinetics of cAMP levels and TGE specific RNA synthesis in PPK cultures
○———○ cAMP levels in TGE infected cells at m.o.i. = 0.1. △———△ cAMP levels in uninfected controls. ●———● cAMP levels in TGE infected cells at m.o.i. = 10. ■———■ cAMP level in cells infected with 0.1 per cent β-propiolactone inactivated TGE virus (M.O.I. — 0.1). □———□ Actinomycin D resistant TGE specific (^{3}H) RNA synthesis in parallel PPK cultures

For this reason, it was decided to investigate the effect of exogenously added cAMP on the TGE specific RNA synthesis. The TGE specific RNA synthesis was measured by the amount of acid insoluble (^{3}H) incorporation in the virally infected cells at different intervals following a 120 minutes pulse of (^{3}H) uridine in the presence of actinomycin D. The detailed procedure has been described elsewhere (6). Briefly, the PPK cells were treated with 2 μg/ml actinomycin for one hour, followed by the addition of the cyclic nucleotide. One hour after the addition of the nucleotide, TGE virus infection was carried out (m.o.i. = 1) for 15 minutes. After washing, fresh media containing appropriate concentrations of actinomycin D and cAMP or dibutyryl cAMP (db-cAMP) were added to each culture and incubated at 37° C. Two hours prior to harvesting each series of culture was pulsed with 2.5 μCi/ml (^{3}H) uridine. The cells were washed, lysed in 0.5 per cent sodium dodecyl sulfate, and precipitated with 5 per cent TCA. The acid insoluble precipitate was collected on nitrocellulose membrane filters, dissolved in Protosol and counted with Aquasol scintillation fluid (New England Nuclear, Chicago, Illinois). Under the experimental conditions used, no significant amount of (^{3}H) uridine incorporation over basal uptake can be detected in uninfected cultures. Figure 2a shows the effect of 1, 10, and 100 μg/ml of cAMP on the kinetics of viral RNA

synthesis. Figure 2b shows that the effect can be duplicated by its analogue—db-cAMP. The db-cAMP is more effective than cAMP at 1 μg level, but not at 10 μg level. At 100 μg/ml concentrations both cAMP and db-cAMP altered the pattern of viral synthesis. When a dose response curve is constructed, it is found that cAMP levels between 1—10 μg proportionally increased peak radioactive uptake at 10 hours post infection but not at concentrations exceeding 10 μg/ml.

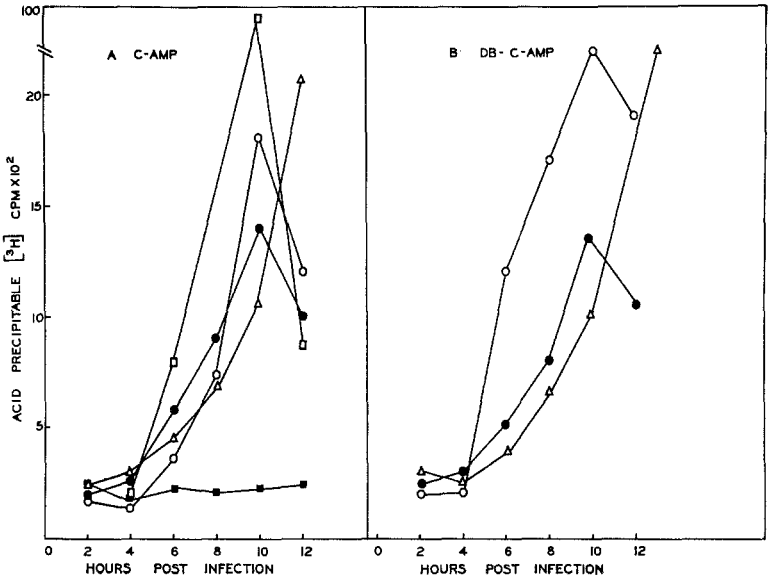

Fig. 2. Effect of exogenous cAMP and DB-cAMP on TGE RNA synthesis
a) Exogenous cAMP
b) Exogenous DB-cAMP
●——● No nucleotide. ■——■ Mock infected with 1 μg/ml cAMP. ○——○ 1 μg/ml. □——□ 10 μg/ml. △——△ 100 μg/ml

In phage infected bacteria, the intracellular cyclic AMP level has been suggested as the regulator which makes the decision between lysogeny and lysis (7). Otten *et al.* (8) have shown that a fall in cAMP level may precede the morphological changes in fibroblasts when they are transformed by Rous Sarcoma virus. The present investigation indicates the possibility that a successful lytic infection may be preceded by a rise in cAMP.

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