Bacteriostatic activity of Emericid

Test organism	Minimum inhibitory concentration (μg/ml)
Staphylococcus aureus (strain 209 P, ATCC 6 538 P)	0.8
Staphylococcus aureus (strain Smith)	2.5
Sarcina lutea (ATCC 9341)	1.9
Streptococcus faecalis (ATCC 8043)	0.5
Streptococcus pyogenes hemolyticus (strain Dig 7, Institut Pasteur)	0.8
Diplococcus pneumoniae (strain Til, Institut Pasteur)	0.2
Neisseria catarrhalis (A 152, Institut Pasteur)	50
Bacillus subtilis (ATCC 6633)	0.8
Bacillus cereus (ATCC 6630)	0.5
Mycobacterium species (ATCC 607)	25
Escherichia coli (ATCC 9637)	> 150
Shigella dysenteriae (Shiga L, Institut Pasteur)	> 150
Salmonella paratyphi A (strain Lacasse, Institut Pasteur	> 150
Salmonella schottmuelleri (paratyphi B; strain Fougenc, Institut Pasteur)	> 150
Proteus vulgaris	> 150
Pseudomonas aeruginosa (strain Bass, Institut Pasteur	> 150

Elemental composition: C 63.2%, H 8.8%, O 24.3%, Na 2.9% is in agreement with the hypothetic molecular formula $C_{44}H_{75}O_{14}Na$; m.W.: 851.06; neutral equivalent with perchloric acid in acetic acid: 840.

According to its physicochemical properties, mainly the peculiar solubility of its sodium salt, emericid seems to belong to the family of ionophore cyclic polyethers[5]. As a matter of fact, proton and 13 C NMR-spectrometry (Figure 2) has shown that emericid owns the same chain of cyclic polyethers as nigericin[6] and grisorixin[7] with only minor differences for the side-substituents. Moreover, attempts to identify emericid with some other products of the family, especially alborixine[8], dianemycin[9], lasalocid[10], lysocellin[11], monensin[12], A 204[13], A 28 695 A and B[14], by thin layer chromatography have been unsuccessful (conditions: spot 100 μg in methylene chloride on a Silicagel Merck F 254 plate; develop with the light phasis of a cyclohexane, ethylacetate, water and butanol 50-50-25-5 mixture; spray with a vanillin 3 g, methanol 100 ml and concentrated sulfuric acid 0.5 ml reagent and heat at 105 °C). Finally, the uniqueness of this compound was substantiated by means of X-ray -crystallography of the silver salt, unraveling its structural formula as shown by Figure 3[15].

The LD_{50} of emericid is 150 mg/kg for the chicken, with a single p.o. administration.

The bacteriostatic activity of emericid against some microorganisms is shown in the Table. The minimum inhibitory concentration determinations were carried out by the dilution method in appropriate medium for each organism and after incubation for 18 h at 37 °C. Emericid is only active in vitro against gram-positive bacteria and practically inactive against any other type of bacteria, yeasts and fungi. In vivo, emericid is inactive against staphylococcal and pneumococcal infections of mice by the oral or s.c. route.

As previously shown[16], emericid is an excellent coccidiostat in chickens; mixed with the feed at 0.006–0.02% levels according to the infecting species of Eimeria, it suppresses mortality and intestinal lesions, reduces oocysts excretion and allows a normal growth of the infected birds; there is no emergence of resistant Eimeria strain during the treatment. Similar results have been obtained in rabbits.

In conclusion, emericid is a new ionophore polycyclic polyether, active in vitro against gram-positive bacteria but devoid of any in vivo antibacterial activity. Its main interest lies in the eradication of coccidiosis in chickens and rabbits at the 0.006–0.02% level in the diet.

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Effect of Cyclic AMP and Theophylline on Phagocytotic Activity of *Tetrahymena pyriformis*

G. Csaba and T. Lantos

Department of Biology, Semmelweis University of Medicine, P.O. Box 95, H-1450 Budapest (Hungary), 3 October 1975.

Summary. Dibutyril cAMP and the cPDE-inhibitor theophylline both enhance the phagocytotic activity of *Tetrahymena*. Theophylline and cAMP-activating histamine are synergistic. It follows that the cAMP-adenylcyclase system functions in the unicellular animal *Tetrahymena*.

Certain forms of hormonal regulation have been shown to be operative in unicellular organisms, although they are seemingly of little consequence at this stage of phylogenesis. Epinephrine[1,2] and serotonin[3] have been shown to be present in *Tetrahymena* at changing concentrations, depending on the functional stage of the protozoon. Also, *Tetrahymena* was found to respond to certain hormones not present in it and not encountered by it under natural conditions. Histamine[4] produced a considerable increase in the phagocytotic activity of the protozoon, whereas

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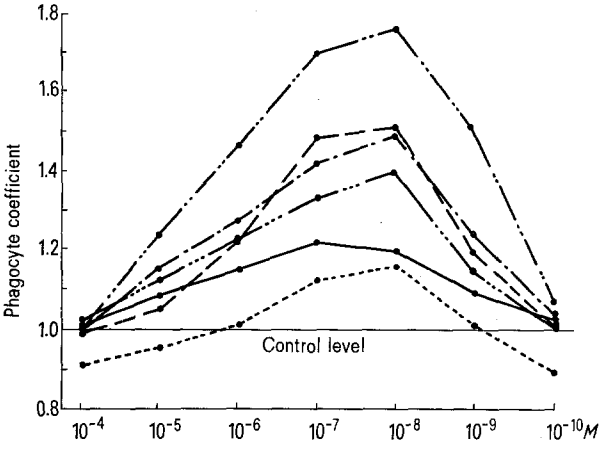
insulin[5] enhanced its glucose uptake. The receptors of *Tetrahymena* are specific enough to differentiate a given hormone from close chemical derivatives[4,6].

Peptide hormones operate mainly via the cyclic AMP-adenylcyclase system, which seems to be present in all living beings[7]. Evidence of the role of cAMP in *Tetrahymena* has been presented by WOLFE[8], ROTHSTEIN and BLUM[9], as well as by CSABA et al.[10], but while the latter authors[9,10] believe it to act as a second messenger, WOLFE[8] does not. Another matter of controversy has been the precise role of cyclic 3′,5′-adenosine monophosphatase diesterase (cPDE) at the lower levels of phylogenesis, which is not well documented[9–13].

In the present experiments, the phagocytic response of *Tetrahymena* to agents that affect the intracellular level of cAMP was tested. Dibutyril cAMP is known to be a highly active cAMP derivative, particularly suited for the experimental study[7] of cAMP action; theophylline is the most active methylxantine inhibitor of cPDE[7], and histamine acts on *Tetrahymena* through its membrane receptors, without entering into the cell, as shown previously[4] in this laboratory.

A *Tetrahymena pyriformis* GL culture grown in 1% Bacto trypton (Difco, Michigan, USA) and 0.05% yeast extract for 2 days at 25 °C was used. 24 h before the experiment the *Tetrahymenae* were isolated from the medium by centrifugation and rendered vacuole-free by incubation in LOSINA-LOSINSKY's solution[14]. The following treatments were carried out: 3 or 10 min exposure to cAMP (N^6, O^2-dibutyril adenosine 3′,5′-cyclic monophosphate Na; Aldrich, Beerse, Belgium); 3 or 10 min exposure to theophylline (Richter, Budapest); 3 or 10 min theophylline treatment followed by 3-min histamine treatment (Reanal, Budapest); no treatment (control series). The concentration range of the test materials was 10^{-4} to 10^{-10} *M*. After pretreatment, Chinese ink diluted in LOSINA-LOSINSKY solution, was added to the *Tetrahymena* lots; after 3 min smears were prepared and were dried rapidly. Each concentration of the applied test materials was tested in 5 replicas in each series and vacuole counts were always determined in 100 animals. The means calculated from readings on 500 protozoa at each concentration level per group were related to the corresponding control reading as 100 to obtain the phagocyte coefficient.



Effect of different combinations of cAMP and theophylline on the phagocyte coefficient of *Tetrahymena*.
—, cAMP 3 min. — — —, cAMP 10 min. · · · ·, Theophylline 3 min. — · · · —, Theophylline 10 min. - · - ·, Theophylline 3 min + histamine 3 min. - · · - · · -, Theophylline 10 min + histamine 10 min.

The experimental results are shown in the Figure. Although the 3-min dibutyril cAMP treatment had little effect, 10-min treatment was sufficient for the development of an action of similar degree to hormonal influence[4]. Theophylline increased the phagocytotic capacity of *Tetrahymena*, but in a lesser degree than cAMP. Its effect greatly depended on the time of treatment, as did the action of cAMP. The phagocyte coefficient obtained on subsequent 3-min exposures to theophylline and histamine did not exceed the value obtained on treatment with histamine alone in earlier studies[4,6], whereas 3-min exposure to histamine following upon 10-min treatment with theophylline enhanced the phagocytotic activity of *Tetrahymena* to a greater degree than any other treatment.

Accordingly, the present findings support the conclusion that the cAMP-adenylcyclase-cPDE system functions in *Tetrahymena*. Another important information emerging from this study is the decisive role of the time factor, the disregard of which can well account for the contradictory results of earlier experimental studies[9,11–13] on hormonal regulation in unicellular animals.

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Cellular Control of the Tick-Borne Virus Antigen Production in Persistently Infected Cell Culture

YU. S. BORISKIN and N. N. BOGOMOLOVA

Moscow Institute for Viral Preparations, 1st Dubrovskaya Street, 15 Moskwa 109088 (USSR), 19 September 1975.

Summary. The influence of inhibition or stimulation of cellular DNA synthesis on tick-borne virus antigen production in persistently infected cell culture was studied. Either mitomycin C or cytosine-arabinoside caused cessation of antigen-containing cell number increase. Stimulation of cellular DNA synthesis by growth medium change increased the level of antigen-containing cells. When HEp-2-Sof culture was synchronized, a correlation was observed between the entrance of cells into DNA synthesis phase and the increase of proportion of antigen-containing cells.

Persistent infection of HEp-2 cell culture by tick-borne encephalitis virus (TBV), designated as HEp-2-Sof[1], has been under study for about 15 years. Some recent findings strongly suggested the participation of cellular genome in the status of chronic infection in this system, namely, 1. stimulation of viral antigen production by 5-bromodeoxyuridine[2], 2. infectious properties of cellular DNA and detection of virus-specific sequences in nuclear DNA by molecular hybridization experiments[3], 3. the lack of DNA excision repair in HEp-2-Sof cells[4].