EPINEPHRINE AND SEROTONIN ACTIVATION OF ADENYL CYCLASE FROM *TETRAHYMENA PYRIFORMIS*

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1. Introduction

Adenyl cyclase has been found in several bacterial species and many higher organisms [1]. The enzyme from Metazoa is activated by sodium fluoride and a wide variety of hormones, whilst the adenyl cyclase of *Brevibacterium liquifaciens* requires pyruvate as a cofactor [2] and that of *Escherichia coli* is inhibited by sodium fluoride [3].

To our knowledge the formation of 3′,5′ cyclic AMP has not previously been shown in protozoa. The findings of Blum [4] that aminophylline increased the glycogen synthetase activity of *Tetrahymena* can be explained by assuming that this drug elevated the level of 3′,5′ cyclic AMP in the cells.

It was thus of interest to examine the adenyl cyclase activity in the ciliate *Tetrahymena pyriformis*. We report here the activation of this enzyme by epinephrine, serotonin and NaF. The abolition of the epinephrine stimulation by propranolol but not phentolamine indicates strongly that the activity of this neurohormone is mediated through a β type adrenergic receptor.

2. Materials and methods

2.1. *Enzyme preparation*

Tetrahymena pyriformis strain W, was grown in a medium containing 2% proteose peptone (Difco), 0.1% yeast extract (Difco) and 5 μg/ml $FeCl_3 \cdot 6H_2O$, in Roux bottles. After 48 hr growth at 28°, cells from 100 ml of the culture were centrifuged at 4300 *g* for 10 min and washed three times in 0.15 M KCl. The cells were then resuspended in 5 ml of 0.25 M sucrose, disrupted in a Branson Sonifier B12 for 1 min at setting 6, and centrifuged at 610 *g* for 10 min. The precipitate containing mostly membranes was suspended in 1.5 ml of 0.24 M Tris buffer pH 7.3 and used as a source of enzymatic activity. All operations were performed at 4°.

2.2. *Assay of adenyl cyclase activity*

The assay system contained: 40 mM Tris-HCl, pH 7.3; 3.3 mM $MgCl_2$; 10 mM caffein; 1 mM [^{3}H]ATP (1 μCi per μmole) and 0.1 ml of enzyme preparation in a final volume of 0.7 ml. The incubation was at 30° for 30 min unless otherwise stated. The reaction was stopped by immersion in a boiling water bath for 3 min. The amount of ^{3}H-labelled cyclic AMP was measured according to a slightly modified procedure of Krishna et al. [5].

Scintillation counting of ^{3}H-labelled cyclic AMP was performed in Bray solution using Packard Tricarb scintillation spectrometer. [^{3}H]ATP (18.3 Ci/mmole) was obtained from Schwartz. Epinephrine, norepinephrine and serotonin were from Sigma. Other materials were of the highest analytical grade available. Protein was estimated by the procedure of Lowry et al. [6], using bovine serum albumin as standard.

3. Results and discussion

The adenyl cyclase activity of *T. pyriformis*, was found to be associated with the membrane fraction obtained after low speed centrifugation of sonicated cells, as has been found in other organisms. The activ-

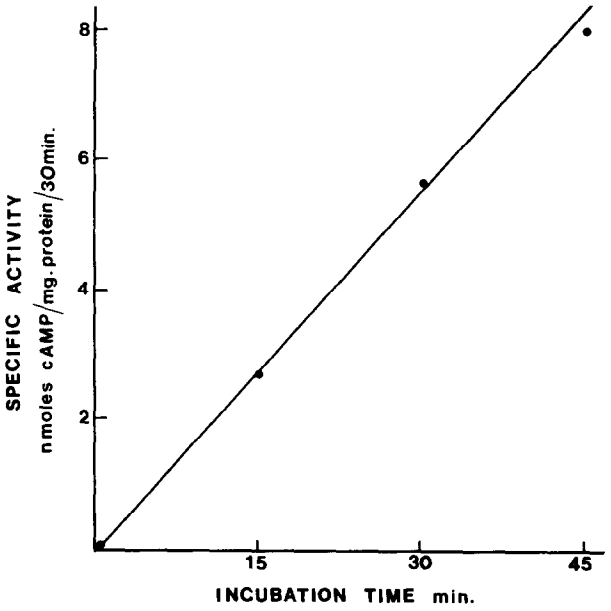

Fig. 1. Time course of adenyl cyclase activity in the presence of 10 mM sodium fluoride.

ity was dependent on the addition of Mg^{2+} and either caffein or aminophylline. The effect of caffein was due to inhibition of a potent phosphodiesterase present in these preparations.

Little activity was observed in the absence of NaF, whilst in its presence a linear response with time was obtained for at least 45 min (fig. 1). Since *T. pyriformis* is known to synthesize epinephrine, norepinephrine

Table 1
Activation of *T. pyriformis* adenyl cyclase.

Addition to and omission from incubation system	Adenyl cyclase specific activity (cyclic AMP formed)
	(nmoles/mg protein/30 min)*
None	1.9
Plus 10 mM NaF	6.3
Plus 1 mM epinephrine	7.8
Plus 1 mM norepinephrine	0.4
Plus 10 mM NaF minus Mg^{2+}	1.9
Plus 10 mM NaF minus caffein	0.6

* Mean values of at least two experiments.

Table 2
Effect of adrenergic blocking agents on stimulation of *T. pyriformis* adenyl cyclase.

Addition to incubation system	Adenyl cyclase specific activity (cyclic AMP formed)*
10 μM Epinephrine	5.1
10 μM Epinephrine + 10 μM propranolol	0.6
10 μM Epinephrine + 10 μM phentolamine	4.9
1 mM NaF	4.7
1 mM NaF + 1 mM propranolol	5.1
1 mM NaF + 1 mM phentolamine	5.6
1 mM Serotonin	6.0
1 mM Serotonin + 1 mM propranolol	5.5
1 mM Serotonin + 1 mM phentolamine	5.9

* Mean values of at least two experiments.

[7] and serotonin [8], these neurohormones were tested for stimulation of cyclase activity. Epinephrine (1 mM) was even more effective than NaF (10 mM) in stimulating the cyclase activity. On the other hand, norepinephrine (1 mM) had no effect, and even seemed to inhibit the unstimulated activity (table 1). The stimulation by epinephrine could be abolished by the β-adrenergic blocking agent, propranolol, but not by the α-adrenergic blocker phentolamine (table 2). Like in other systems described propranolol had no effect on the stimulation produced by fluoride. The lower activity in the presence of epinephrine and propranolol than in the absence of any additions indicates that endogenous epinephrine was present in the preparations.

The cyclase activity was also increased by serotonin; its stimulation was lower than that caused by epinephrine but higher than that of fluoride. The comparison of the dose-response effect of NaF, epinephrine and serotonin is presented in fig. 2. The serotonin activation was not inhibited by either propranolol or by phentolamine at equimolar concentrations. Serotonin was shown to enhance adenyl cyclase mediated reactions in such diverse organisms as the liver fluke [9] and rabbit [10], but no direct effect on the cyclase *in vitro* was reported.

The inhibition of the growth of *Tetrahymena* by both α and β adrenergic blocking agents was shown by Bloom [11]. However, on the basis of these experiments it was not possible to decide whether the

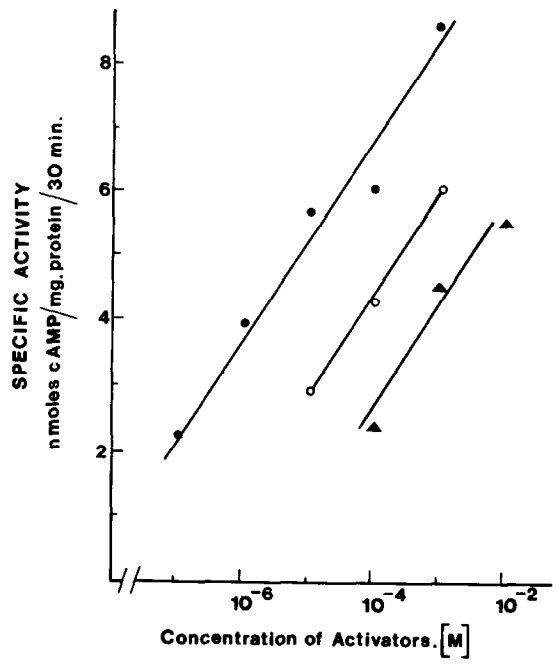

Fig. 2. Effect of the concentration of stimulatory substances on the activity of adenyl cyclase of *T. pyriformis*. (● - ●) Epinephrine; (○ - ○) serotonin; (▲ - ▲) NaF.

inhibition was related to adrenergic sites or to other growth controlling systems [12]. Our results support strongly the hypothesis that adenyl cyclase in *T. pyriformis* is activated by epinephrine through a β-type receptor. Thus growth inhibition by propranolol can be related to distorted metabolic control due to inhibition of the β-receptor.

These observations may allow us to assign a function to the presence of epinephrine and serotonin in *Tetrahymena*, viz. as activators of cAMP synthesis. As pointed out by Janakidevi et al. [7], the ultimate role of these substances may be in the regulation of polysaccharide reserve, particularly as this ciliate has a large store of glycogen. However, since cyclic AMP is important in many reactions, the activation of its synthesis probably has also other functions.

The relative ease of growing *T. pyriformis* and a uniform cell population under defined conditions, should facilitate greatly the purification and further characterization of the β-like adrenergic receptor.

References

[1] J-P. Jost and H.V. Rickenberg, Ann. Rev. Biochem. 40 (1971) 741.
[2] M. Hirata and O. Hayaishi, Biochem. Biophys. Acta 149 (1967) 1.
[3] M. Tao and F. Lipmann, Proc. Natl. Acad. Sci. U.S. 63 (1969) 86.
[4] J.J. Blum, Arch. Biochem. Biophys. 137 (1970) 65.
[5] G. Krishna, B. Weiss and B.B. Brodie, J. Pharmacol. Exp. Ther. 163 (1968) 379.
[6] O.H. Lowry, N.J. Rosenbrough, A.L. Farr and R.J. Randall, J. Biol. Chem. 193 (1951) 265.
[7] K. Janakidevi, V.C. Dewey and G.W. Kidder, J. Biol. Chem. 241 (1966) 2576.
[8] K. Janakidevi, V.C. Dewey and G.W. Kidder, Arch. Biochem. Biophys. 113 (1966) 758.
[9] D.B. Stone and T.E. Mansour, Mol. Pharmacol. 3 (1967) 161.
[10] S. Kakiuchi and T.W. Rall, Mol. Pharmacol. 4 (1968) 367.
[11] J.J. Blum, Proc. Natl. Acad. Sci. U.S. 58 (1967) 81.
[12] J.J. Blum, J. Protozool. 16 (1969) 317.